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The effect of adiposity on differences in carotid plaque burden in studies conducted in Norway and Russia: a cross-sectional analysis of two populations at very different risk of cardiovascular mortality

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Key words

Epidemiology, Carotid atherosclerosis, Adiposity, Russian Federation, Europe

Word counts

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Abstract

Objectives: Large differences exist in the burden of cardiovascular disease (CVD) between Russia and Western European countries including Norway. Obesity prevalence may contribute to the differences. We investigated whether difference in the level of adiposity could explain inter-country differences in the burden of carotid plaque a measure of atherosclerosis in the populations.

Design: Cross-sectional analysis.

Setting: We used population-based cross-sectional Know Your Heart (KYH) study in Russia and the Tromsø 7 study (Tromsø 7) in Norway.

Participants: 3262 and 1800 men and women aged 40-69 years in KYH and Tromsø 7, respectively.

Primary and secondary outcome: The presence of carotid plaques and plaque score assessed using ultrasound.

Results: A positive association between carotid plaque burden and adiposity was found (OR of having at least one plaque per SD in WHR 1.18 (95% CI 1.06, 1.31) for men; 1.15 (1.06, 1.25) for women)) adjusted for age, smoking and education. These effects did not differ between the two studies. However, neither adiposity nor CVD risk factors (smoking, systolic blood pressure, cholesterol, glycosylated haemoglobin) explained the higher carotid plaque burden in KYH compared to Tromsø 7.

Conclusion: Adiposity, especially abdominal adiposity, is a risk factor for carotid plaque in Russia and Norway, although neither adiposity nor established CVD risk factors explained the higher plaque burden in Russia. To reduce the CVD burden in Russia, beyond prevention and treatment of adiposity, further research is required to understand why Russia has a high burden of atherosclerosis.

Strengths and limitations of this study

- This is the first study to compare adiposity level, carotid plaque burden, and its association with adiposity between Russia and Western European country with low CVD mortality.
- The use of two substantial population-based studies with similar study period and study protocols enabled us to make a direct comparison of two populations.
- Waist circumference was measured at different measurement sites between the two studies.
- We did not assess visceral adiposity or body composition.

Introduction

The mortality rate from cardiovascular disease (CVD) has been decreasing for many years in Western Europe, and more recently in Eastern Europe (1). However, rates vary substantially between countries, with Russia having one of the highest CVD mortality rates (2), although it has been declining since 2005 (3). In 2012-16, the CVD mortality rate at working-ages in Russia was eight times higher than that in Norway (4). These premature deaths contribute to the relatively low life expectancy for such an industrialised country. However, the reasons for this very high CVD burden in Russia remain unclear (4).

The increase in obesity over the past decades is a growing concern worldwide, including in Russia and countries of Western Europe (5), and has an effect on mortality levels (6). In addition to general obesity, however, the extent of abdominal obesity is likely to be important as there is evidence that it is more strongly associated with CVD events than general adiposity assessed using body mass index (BMI) (7-9). However, data on population-levels of abdominal obesity (such as waist-hip ratio (WHR)) is far less common than for BMI, in Russia as well as in other countries.

Carotid plaque, representing an advanced stage of atherosclerosis, is predictive of future CVD events (10). Carotid atherosclerosis may be easily and reliably detected using an ultrasound examination making carotid plaque a good surrogate marker of atherosclerotic CVD burden in large-scale epidemiological studies.

We used data from two studies from general populations with very different CVD mortality in Europe: Know Your Heart (KYH) study in Russia and Tromsø Study seventh survey (Tromsø 7) in Norway. Our aims were: 1) to compare general and abdominal adiposity levels and the burden of carotid plaque in Russia with those in Norway, a low CVD mortality country; 2) to investigate the association between general/abdominal adiposity and carotid plaque in both populations; and 3) to investigate whether general/abdominal adiposity or other factors can explain difference in carotid plaque burden between the two populations.

Methods

Study design and participants

KYH is a population-based cross-sectional study of 4500 women and men aged 35-69 years conducted between 2015 and 2017 in two Russian cities: Novosibirsk and Arkhangelsk. The details of KYH have been described elsewhere (4). Briefly, participants were recruited from a random sample of the population stratified by age and gender, derived from the list of the Territorial Health Insurance Funds. Trained interviewers visited the addresses on the list and identified residents of the target age and sex. Information on socio-demographic characteristics, CVD risk factors and medical history were collected using structured questionnaires completed on tablet computers. At the end of the

interview participants were invited to have a comprehensive examination including anthropometric measurement, blood sampling, and carotid ultrasound one or two weeks later. Response rates for initial interview were 53% and 27% in Arkhangelsk and Novosibirsk, respectively. Of those interviewed 89% attended the subsequent medical examination. All participants of the medical examination provided signed informed consent.

The Tromsø Study is an ongoing population-based study in Tromsø municipality, North Norway, and consists of seven surveys from 1974-2016 (11). In the seventh wave (Tromsø 7), all residents in Tromsø aged 40 years and older were invited to participate. The questionnaires was completed, a brief physical examination was carried out, and biological samples were taken. A random sample including previous participants were invited to a second visit to undergo more comprehensive medical examinations. A total of 21083 attended the first visit and the response rate was 65%. 4153 participants were invited to a carotid ultrasound examination and 2974 (71.6%) attended.

Participants aged between 40-69 years (n=5782) were eligible for the present study. We excluded participants with missing data on all adiposity measures (n=42), and potential confounders and mediators (n=678), leaving 3262 participants from KYH (57% women) and 1800 from Tromsø 7 (55% women) for the analyses.

Assessment of anthropometric measures and other CVD risk factors

In both studies, height and weight were assessed by trained staff using standard methods (see supplementary material). BMI was calculated by dividing weight in kilograms by squared height in meters. Waist circumference (WC) was measured at a different site in the two studies: in KYH WC was measured at the narrowest part of the trunk to the nearest millimetre using a tape measure while in Tromsø 7 WC was measured at the level of the umbilicus. Hip circumference was measured at the widest part in both studies. To ensure WC was comparable between the two studies, WC in Tromsø 7 was converted to the narrowest waist using a conversion equation by Mason et al. (12). Among anthropometric measures of abdominal adiposity WHR was selected because it has been found to be strongly associated with CVD events (8, 9, 13).

Information on age (5-year categories), smoking (current smoker, ex-smoker, never-smoker), educational attainment (higher education: Yes/No), and medical history of diabetes (DM) (Yes/No) were collected through face-to-face interview in KYH and self-administered questionnaire in Tromsø 7. The assessment of systolic blood pressure (SBP) and other mediators are described elsewhere (4).

Ultrasound examination

The examination protocols were aligned between the two studies. Both carotid arteries were scanned for carotid plaques in the common carotid artery (CCA), bifurcation and internal carotid artery (ICA) using a Vivid Q (GE Health care) with 6~13 MHz linear transducers in KYH and Vivid 7 (GE Health

care) with a linear 12 MHz transducer in Tromsø 7. Carotid plaque was defined according to the Mannheim Consensus as a focal structure encroaching into the arterial lumen by at least 0.5 mm, or having a thickness ≥50% greater than the surrounding intima-media thickness (IMT), or IMT >1.5 mm as measured from the media-adventitia interface to the intima-lumen interface (14).

To evaluate the burden of carotid plaque, we created a cumulative plaque score by assigning a score of one for the presence of one or more plaques in each of the six carotid segments (CCA, bifurcation, and ICA of each carotid artery) with a maximum possible score of six for each individual.

Statistical methods

Analyses were conducted stratifying by sex a priori. Two outcome measures were used: the presence of plaques as a binary outcome and plaque score. As exposures, BMI and WHR were used to represent general and abdominal adiposity, respectively. To enable direct comparison of the magnitude of the effects of BMI and WHR, sex-specific adiposity z-scores standardised to Tromsø 7 participants were created by subtracting the mean and dividing by the standard deviation (SD) of each measure in Tromsø 7.

Age, smoking, and education were considered a priori confounders while SBP, HDL cholesterol, LDL cholesterol, glycosylated haemoglobin (HbA1c) and medical history of DM were considered as potential mediators. Sex-specific linear and logistic regression models were used to investigate the associations of each adiposity with plaque score and presence of plaques respectively. A series of models were fitted that were specific to each sex and study (4 in all). Model 1 adjusted for age (5-year age-groups). Model 2, our main model to elucidate the association between adiposity and plaque burden, further adjusted for potential confounders. Model 3 further adjusted for potential mediators to see to what extent the association was mediated by these factors. These analyses were conducted using the data from each study and the pooled data from the two studies after checking for interaction with study. This was done by adding an interaction term between study and adiposity using pooled data: testing for statistical significance using likelihood ratio tests for logistic regression and Wald tests for linear regression.

Finally, to estimate the difference in plaque burden between the two studies, we applied a similar set of models as already described to the pooled data using a binary indicator for study. The associations between each study and plaque burden (the presence of plaques, plaque score) were estimated using logistic and linear regression, respectively. To look at adjusted difference in plaque burden between the two studies, three similar models adjusted for age, confounders, and mediators, were applied without adjustment for adiposity. We then separately added each adiposity measure to these models to estimate the effect of adiposity on between-study difference in carotid plaque burden.

STATA version 15 (Stata Corp) was used for all the analyses.

Ethical approval for the KYH study was received from the ethics committees of the London School of Hygiene & Tropical Medicine, Novosibirsk State Medical University, the Institute of Internal and Preventative Medicine, Novosibirsk and the Northern State Medical University, Arkhangelsk . Ethical approval for Tromsø 7 was obtained the Regional Committee for Medical and Health Research Ethics (reference number 2014/940).

Results

Baseline characteristics of participants

Table 1 shows participants' baseline characteristics. The age-adjusted prevalence of current smoking in men was much higher in KYH than Tromsø 7 but similar for women. However, female never smokers made up two-thirds in KYH but just over a third in Tromsø 7. Mean SBP was considerably higher in KYH than in Tromsø 7.

Adiposity

Both BMI and WHR were higher for women in KYH than those in Tromsø 7. Adiposity z-scores for BMI and WHR for the KYH women standardised to the Tromsø 7 population adjusted for age were 0.58 (95% CI: 0.49, 0.68) and 0.84 (95% CI: 0.76, 0.92), respectively. However, adiposity did not differ between men in the two studies.

Prevalence of carotid plaques

The prevalence of carotid plaques and the mean plaque score increased with age in both women and men (Table 2). The burden of plaques was consistently higher in KYH than Tromsø 7 in both sexes.

The association between carotid plaque burden and adiposity: a pooled analysis of the two studies

Table 3 shows the odds ratios for having at least one carotid plaque per 1SD increase in each adiposity measure by sex from the pooled analysis. The two adiposity measures were not adjusted for each other. After adjustment for confounders (Model 2), there was evidence of association between all adiposity measures and the presence of plaques except for BMI in women. WHR showed larger standardized odds ratios (women 1.15 95% CI: 1.06, 1.25, men 1.18 95% CI: 1.06, 1.31) than BMI. After further adjustment for cardio-metabolic mediators (Model 3), all odds ratios decreased substantially.

Table 4 shows the difference in plaque score per 1SD increase in each adiposity measure. In women, adiposity was associated with an increase in plaque score. Again WHR showed a larger effect size (increase per 1SD change 0.109 95% CI: 0.070, 0.147) than BMI. Additional adjustments for cardio-metabolic mediators reduced both effect sizes substantially. For men, there was no evidence of an association of BMI and WHR with plaque score.

Tests for interaction between study and adiposity were not statistically significant except for the association between BMI and the presence of plaque in women and that between WHR and the plaque score in women, suggesting that there is little evidence that the association between adiposity and plaque burden differs between the two studies (Supplementary table 1, Supplementary figure 1A, 1B).

Between-study differences in carotid plaque burden and the effect of adiposity

Figure 1 compares the carotid plaque burden between the two studies with and without adjustment for adiposity. Without adjustment for adiposity measures, the odds ratio for having at least one plaque in KYH compared to Tromsø 7 was 1.97 (95% CI: 1.62, 2.38) in women and 2.78 (95% CI: 2.21, 3.49) in men (Figure 1a 2, Supplementary table 2A Model 2). Further adjustment for BMI or WHR separately had only a small effect on this odds ratio for both men and women (Figure 1a 3,4). The between-study difference remained large and statistically significant after further adjustment for cardio-metabolic mediators (Figure 1a 5, Supplementary table 2A Model 3).

Similarly, without adjustment for adiposity, participants in KYH had a higher mean plaque score than those in Tromsø 7 by 0.51 (95% CI: 0.42, 0.60) for women and 0.89 (95% CI: 0.77, 1.00) for men; these estimates decreased slightly for women and hardly changed at all for men with further adjustment for adiposity (Figure 1b 2, Supplementary table 2B). The between-study difference remained significant after further adjustment for cardio-metabolic mediators (Figure 1b 5, Supplementary table 2B Model 3).

Discussion

There was evidence of positive associations between adiposity, especially abdominal adiposity, and carotid plaque burden, but no convincing evidence that the strength of these associations differed between the two studies. These associations were largely mediated by cardio-metabolic CVD risk factors. However, neither adiposity nor the confounders and potential mediators explained the substantially greater burden of plaque in the KYH study in Russia compared to the Tromso 7 study in Norway. To the best of our knowledge, this is the first study to directly investigate the role of adiposity in high CVD burden in a general population in Russia in comparison with another country.

Given the similar adiposity level between KYH and Tromsø 7 among men, it is not surprising that higher plaque burden in men in KYH is not explained by adiposity. However, even among women whose adiposity level was considerably higher in KYH than Tromsø 7, the adjustment for adiposity had little impact on the inter-study difference in carotid plaque burden. Furthermore, additional adjustment for CVD and metabolic risk factors such as smoking, systolic blood pressure and cholesterol level slightly reduced this inter-study difference, but the between-study difference remained for both men and women, suggesting that there are other determinants of higher carotid

plaque burden in a population in KYH. This is consistent with previous studies conducted 20 or more years ago, showing that differences in traditional CVD risk factors did not fully explain the high CVD burden in Russia compared to Western European countries (15, 16). More advanced subclinical atherosclerosis in participants in KYH compared to Tromsø 7 is in keeping with the higher CVD mortality rate in Russia than Western European countries (4, 17).

The development of coronary artery disease and atherosclerotic plaque is a gradual process that occurs across the lifecourse (18). The extent to which single cross-sectional measurements of risk factors such as blood pressure and smoking can capture the full impact of these risk factors on the burden of carotid plaque is therefore questionable. In making the sort of comparisons between populations that are the focus of this paper therefore, it could be that we are underestimating the potential contribution of these risk factors to differences in plaque burden, particularly if risk factor profiles have been changing.

One potential determinant of high CVD risk in Russia that we have not included is alcohol which has been shown to be related to mortality from cardiovascular disease(19). Vikhireva et al. added hazardous alcohol consumption to the high-risk version of SCORE to see whether this modified model improved prognostic performance of SCORE for future CVD events in the Russian population. However, this modification did not improve the prediction of CVD events (20) although the study had limited follow-up and relatively small numbers of events. Moreover, it excluded as an outcome alcoholic cardiomyopathy that contributes to the high CVD mortality in Russia (21) involving processes other than atherosclerosis (22). Differences in treatment and access to the medical facilities between Western Europe and Russia is likely to partly account for the higher CVD mortality rates in Russia, but it is unlikely that differences in treatment could account for the differences in subclinical atherosclerosis in a population-based samples. Furthermore, the treatment and access to appropriate medical care have been improving rapidly in Russia, especially in large cities, so this is likely to be a less important factor in the future (23, 24)[ ]. Identification of the determinant(s) of advanced subclinical atherosclerosis in Russia will be important to target interventions to reduce CVD burden.

There was evidence of positive associations between adiposity and plaque burden in both studies emphasising the importance of the control of adiposity, especially abdominal adiposity, to curb the CVD burden in both countries. The prevalence of obesity in Russia has been increasing (5), with the notably high level among women being of particular concern (5). Another important implication of our findings is the importance of the control of cardiovascular and cardio-metabolic mediators. The associations between adiposity and carotid plaque burden were largely mediated by SBP, cholesterol level and HbA1c. The effective control of these traditional risk factors will mitigate the negative effect of adiposity.

This study is the first, to our knowledge, to bring together data from a high and relatively low CVD mortality country to investigate the association of adiposity with carotid plaque, and also the extent to which this can explain differences in the burden of carotid plaque between the two populations. Moreover, this was done using ultrasound examination protocols that were aligned between the two studies. No previous studies have compared imaging of atherosclerotic changes in general populations between Russia and Western countries. However, our investigation has some limitations. First, the anthropometric measures we used are crude measures of visceral adiposity. However, estimation of visceral adipose tissue using MRI and CT is resource-demanding and logistically difficult in large epidemiological studies. Second, WC was measured differently between the two studies. Although the conversion of WC was made using a conversion equation, this did not allow for individual variability. Standardisation of the protocol of WC measurement would be important in future studies. Third, we did not include alcohol in our regression models, although it is likely to play an important role in CVD mortality in Russia (25-28). Finally, as always, caution must be exercised in generalising to the national situation the results we have obtained from the two cross-sectional studies of selected groups in two cities in Russian and one in Norway city.

Overall, our findings have two implications with respect to tackling the high CVD burden in Russia. First, although adiposity failed to explain higher plaque burden in Russia compared to Norway, adiposity, especially abdominal adiposity, appeared to contribute to an increase in carotid plaque burden through cardio-metabolic mediators such as blood pressure and cholesterol. The reduction of adiposity level will be important to avoid further CVD burden in addition to the control of cardio-metabolic mediators. Second, our findings suggest that there are other unidentified risk factors that determine the higher carotid plaque burden in Russia compared to Norway. Further studies will be needed to identify them.

References

1. Ezzati M, Obermeyer Z, Tzoulaki I, Mayosi BM, Elliott P, Leon DA. Contributions of risk factors and medical care to cardiovascular mortality trends. Nat Rev Cardiol. 2015;12(9):508-30.
2. Timmis A, Townsend N, Gale C, Grobbee R, Maniadakis N, Flather M, et al. European Society of Cardiology: Cardiovascular Disease Statistics 2017. Eur Heart J. 2018;39(7):508-79.
3. Grigoriev P, Meslé F, Shkolnikov VM, Andreev E, Fihel A, Pechholdova M, et al. The recent mortality decline in Russia: Beginning of the cardiovascular revolution? 2014;40(1):107-29.
4. Cook S, Malyutina S, Kudryavtsev A, Averina M, Bobrova N, Boytsov S, et al. Know Your Heart: Rationale, design and conduct of a cross-sectional study of cardiovascular structure, function and risk factors in 4500 men and women aged 35-69 years from two Russian cities, 2015-18 [version 1; referees: 1 approved, 1 approved with reservations]. Wellcome Open Research. 2018;3(67).
5. NCD Risk Factor Collaboration. Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. Lancet. 2016;387(10026):1377-96.
6. Vidra N, Trias-Llimos S, Janssen F. Impact of obesity on life expectancy among different European countries: secondary analysis of population-level data over the 1975-2012 period. BMJ Open. 2019;9(7):e028086.
7. Peters SAE, Bots SH, Woodward M. Sex Differences in the Association Between Measures of General and Central Adiposity and the Risk of Myocardial Infarction: Results From the UK Biobank. J Am Heart Assoc. 2018;7(5).
8. Emdin CA, Khera AV, Natarajan P, Klarin D, Zekavat SM, Hsiao AJ, et al. Genetic Association of Waist-to-Hip Ratio With Cardiometabolic Traits, Type 2 Diabetes, and Coronary Heart Disease. Jama. 2017;317(6):626-34.
9. Dale CE, Fatemifar G, Palmer TM, White J, Prieto-Merino D, Zabaneh D, et al. Causal Associations of Adiposity and Body Fat Distribution With Coronary Heart Disease, Stroke Subtypes, and Type 2 Diabetes Mellitus: A Mendelian Randomization Analysis. Circulation. 2017;135(24):2373-88.
10. Inaba Y, Chen JA, Bergmann SR. Carotid plaque, compared with carotid intima-media thickness, more accurately predicts coronary artery disease events: a meta-analysis. Atherosclerosis. 2012;220(1):128-33.
11. Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njolstad I. Cohort profile: the Tromso Study. Int J Epidemiol. 2012;41(4):961-7.
12. Mason C, Katzmarzyk PT. Variability in waist circumference measurements according to anatomic measurement site. Obesity (Silver Spring). 2009;17(9):1789-95.
13. O'Donnell MJ, Chin SL, Rangarajan S, Xavier D, Liu L, Zhang H, et al. Global and regional effects of potentially modifiable risk factors associated with acute stroke in 32 countries (INTERSTROKE): a case-control study. Lancet. 2016;388(10046):761-75.
14. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, et al. Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011. Cerebrovasc Dis. 2012;34(4):290-6.
15. Kuulasmaa K, Tunstall-Pedoe H, Dobson A, Fortmann S, Sans S, Tolonen H, et al. Estimation of contribution of changes in classic risk factors to trends in coronary-event rates across the WHO MONICA Project populations. Lancet. 2000;355(9205):675-87.
16. Averina M, Nilssen O, Brenn T, Brox J, Kalinin AG, Arkhipovsky VL. High cardiovascular mortality in Russia cannot be explained by the classical risk factors. The Arkhangelsk Study 2000. European Journal of Epidemiology. 2003;18(9):871-8.
17. Townsend N, Wilson L, Bhatnagar P, Wickramasinghe K, Rayner M, Nichols M. Cardiovascular disease in Europe: epidemiological update 2016. Eur Heart J. 2016.

18. Rose G. Incubation period of coronary heart disease. Br Med J (Clin Res Ed). 1982;284(6329):1600-1.
19. Leon DA, Shkolnikov VM, McKee M, Kiryanov N, Andreev E. Alcohol increases circulatory disease mortality in Russia: acute and chronic effects or misattribution of cause? Int J Epidemiol. 2010;39(5):1279-90.
20. Vikhireva O, Kubinova R, Malyutina S, Pajak A, Simonova G, Bobak M, et al. Inclusion of hazardous drinking does not improve the SCORE performance in men from Central and Eastern Europe: the findings from the HAPIEE cohorts. BMC Public Health. 2014;14:1187.
21. Manthey J, Probst C, Rylett M, Rehm J. National, regional and global mortality due to alcoholic cardiomyopathy in 2015. Heart. 2018;104(20):1663-9.
22. Leon DA, Shkolnikov VM, Borinskaya S, Casas JP, Evans A, Gil A, et al. Hazardous alcohol consumption is associated with increased levels of B-type natriuretic peptide: evidence from two population-based studies. Eur J Epidemiol. 2013;28(5):393-404.
23. Timonin S, Kontsevaya A, McKee M, Leon DA. Reducing geographic inequalities in access times for acute treatment of myocardial infarction in a large country: the example of Russia. Int J Epidemiol. 2018;47(5):1594-602.
24. Kontsevaya A, Sabgaida T, Ivanova A, Leon DA, McKee M. How has the management of acute coronary syndrome changed in the Russian Federation during the last 10 years? Health Policy. 2017;121(12):1274-9.
25. The burden of disease in Russia from 1980 to 2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2018;392(10153):1138-46.
26. Feigin VL, Roth GA, Naghavi M, Parmar P, Krishnamurthi R, Chugh S, et al. Global burden of stroke and risk factors in 188 countries, during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet Neurol. 2016;15(9):913-24.
27. Zaridze D, Brennan P, Boreham J, Boroda A, Karpov R, Lazarev A, et al. Alcohol and cause-specific mortality in Russia: a retrospective case-control study of 48,557 adult deaths. Lancet. 2009;373(9682):2201-14.
28. Leon DA, Saburova L, Tomkins S, Andreev E, Kiryanov N, McKee M, et al. Hazardous alcohol drinking and premature mortality in Russia: a population based case-control study. Lancet. 2007;369(9578):2001-9.

Footnotes

Contributors: YI undertook the analyses and drafting of the manuscript. CF advised on statistical methods and analysis. DL and CF provided ongoing guidance during drafting. EBM, AR, SM provided guidance on accessing and understanding the Tromsø 7 and KYH. All authors read and commented on drafts of the paper and approved the final manuscript.

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Provenance and peer review: Not commissioned; externally peer reviewed

Data availability statement: Metadata is available for the Tromsø study at http://tromsoundersokelsen.uit.no/tromso/ and for the Know Your Heart study at https://metadata.knowyourheart.science/. The data used in these analyses cannot be placed in the public domain because of ethical and data protection restrictions.

Table 1: Participant characteristics in Know Your Heart (KYH) and Tromsø 7 (T7)*

	Men			Women		
	KYH	**Tromsø 7**	**Comparison† KYH vs T7**	**KYH**	**Tromsø 7**	**Comparison† KYH vs T7**
N	1389	811		1873	989	
Age years	56 (48-63)	61 (52-66)		55 (48-63)	60 (52-65)	
Anthropometric measure			**Difference (95% CI)**			**Difference (95% CI)**
Height cm	174.7 (6.6)	177.9 (6.7)	-3.9 (-4.4, -3.3)	161.1 (6.3)	164.7 (6.3)	-4.1 (-4.6, -3.6)
Weight kg	84.5 (15.5)	87.5 (12.9)	-3.6 (-4.9, -2.3)	74.5 (16.1)	71.4 (12.9)	3.4 (2.3, 4.6)
BMI kg/m^2	27.6 (4.6)	27.7 (3.7)	0.0 (-0.3, 0.4)	28.8 (6.2)	26.4 (4.7)	2.7 (2.3, 3.1)
WHR[a]	0.95 (0.07)	0.95 (0.07)	-0.00 (-0.00, 0.01)	0.84 (0.08)	0.79 (0.07)	0.06 (0.05, 0.06)
Potential confounders			**Odds Ratio (95% CI)**			**Odds Ratio (95% CI)**
Never-smoker (%)	351 (25.3)	316 (39.0)	(ref)	1304 (69.6)	383 (38.7)	(ref)
Ex-smoker (%)	518 (37.3)	377 (46.5)	1.3 (1.1, 1.7)	285 (15.2)	451 (45.6)	0.2 (0.1, 0.2)
Current smoker (%)	520 (37.4)	118 (14.6)	4.0 (3.1, 5.1)	284 (15.2)	155 (15.7)	0.5 (0.4, 0.6)
Higher education (%)	478 (34.3)	388 (47.8)	0.6 (0.5, 0.7)	701 (37.4)	495 (50.1)	0.5 (0.4, 0.6)
Potential mediators			**Difference (95% CI)**			**Difference (95% CI)**
SBP mmHg	138.6 (19.9)	132.8 (17.9)	7.3 (5.6, 8.9)	129.8(19.6)	126.4(19.4)	5.7 (4.3, 7.2)
Total cholesterol mmol/l	5.38 (1.13)	5.42 (1.05)	-0.06 (-0.16, 0.04)	5.68 (1.17)	5.62 (1.04)	0.13 (0.04, 0.21)
Triglycerides mmol/l	1.35 (0.95, -1.92)	1.50 (1.00, -2.10)	-0.05 (-0.15, 0.06)	1.23 (0.89,-1.77)	1.10 (0.80, -.60)	0.18 (0.11, 0.26)
HDL cholesterol mmol/l	1.32 (0.33)	1.41 (0.39)	-0.09 (-0.12, -0.06)	1.55 (0.36)	1.78 (0.49)	-0.24 (-0.27, -0.20)
LDL cholesterol mmol/l	3.66 (0.90)	3.63 (0.99)	0.01 (-0.07, 0.09)	3.80 (0.95)	3.60 (0.96)	0.27 (0.19, 0.34)
HbA1c (%)	5.60 (0.84)	5.74 (0.57)	-0.08 (-0.14, -0.01)	5.57 (7.69)	5.67 (0.51)	-0.04 (-0.09, 0.01)
			Odds Ratio (95%CI)			**Odds Ratio (95%CI)**
Diabetes mellitus (%)	93 (6.7)	47 (5.8)	1.4 (1.0, 2.1)	182 (9.7)	52 (5.3)	2.5 (1.8, 3.5)

Data are presented as percentages for binary variables and as mean values (SD) continuous variables, except for age, triglycerides which are presented as median (inter-quintile range (IQR)).

*Analyses were restricted to participants aged between 40 and 69 years with information on all covariates.

†all comparisons age-adjusted

[a]WC in Tromsø 7 assessed at the level of the umbilicus was converted to the narrowest WC so that it can be comparable with WC in KYH. WHR in T7 are calculated using converted WC.

BMI: body mass index, HDL: high-density lipoprotein, LDL: low-density lipoprotein, WC: waist circumference, WHR: waist-to-hip ratio

Table 2. The prevalence of carotid plaques and plaque score according to study and sex

	Men			Women		
	KYH	**Tromsø 7**	**Comparison KYH vs T7[a]**	**KYH**	**Tromsø 7**	**Comparison KYH vs T7[a]**
N	1389	811		1873	989	
Prevalence n (%)			**Odds Ratio (95% CI)**			**Odds Ratio (95% CI)**
All age	1050 (75.6)	499 (61.5)	3.2 (2.6, 4.0)	1043 (55.7)	478 (48.3)	1.9 (1.6, 2.3)
40-49	212/394 (53.8)	38/148 (25.7)	3.5 (2.3, 5.4)	174/570 (30.5)	40/189 (21.2)	1.7 (1.1, 2.5)
50-59	340/450 (75.6)	104/191 (54.5)	2.7 (1.9, 4.0)	328/605 (54.2)	112/273 (41.0)	1.7 (1.3, 2.3)
60-69	498/545 (91.4)	357/472 (75.6)	3.5 (2.4, 5.1)	541/698 (77.5)	326/527 (61.9)	2.1 (1.7, 2.7)
Plaque score mean (SD)			**Difference (95% CI)**			**Difference (95% CI)**
All age	1.9 (1.6)	1.2 (1.1)	1.0 (0.9, 1.2)	1.1 (1.3)	0.8 (0.9)	0.5 (0.4, 0.6)
40-49	1.0 (1.2)	0.3 (0.6)	0.6 (0.5, 0.8)	0.5 (0.8)	0.3 (0.6)	0.2 (0.1, 0.3)
50-59	1.8 (1.5)	0.9 (1.0)	0.9 (0.7, 1.2)	1.0 (1.2)	0.6 (0.8)	0.4 (0.3, 0.6)
60-69	2.7 (1.6)	1.5 (1.2)	1.2 (1.1, 1.4)	1.7 (1.4)	1.1 (1.0)	0.7 (0.5, 0.8)

[a]Adjusted for categorical age (5-year interval)

Table 3: Odds ratios for having at least one plaque per 1 SD increase in each adiposity measure: pooled results from the two studies

	Model 1 OR (95% CI)	p-value	Model 2 OR (95% CI)	p-value	Model 3 OR (95% CI)	p-value
Men (n=2200)						
st BMI	1.12 (1.02, 1.23)	0.02	1.13 (1.03, 1.24)	0.01	1.01 (0.91, 1.12)	0.83
st WHR	1.21 (1.08, 1.34)	0.001	1.18 (1.06, 1.31)	0.003	1.05 (0.93, 1.18)	0.46
Women (n=2862)						
st BMI	1.06 (0.99, 1.14)	0.08	1.06 (0.99, 1.13)	0.09	0.94 (0.87, 1.01)	0.08
st WHR	1.20 (1.11, 1.30)	<0.001	1.15 (1.06, 1.25)	0.001	1.00 (0.91, 1.09)	0.94

St BMI: body mass index z-score, st WHR:waist-to-hip ratio z-score, OR: odds ratio, 95%CI: 95% confidence interval
Model 1: adjusted for categorical age (5-year) and study, Model 2: adjust for variables in Model 1 plus potential confounders (smoking and education), Model 3: adjusted for variables in Model 2 plus potential mediators (systolic blood pressure, HDL cholesterol, LDL cholesterol, glycated haemoglobin, medical history of diabetes)

Table 4: Difference in plaque score per 1 SD increase in each adiposity measure: pooled results from the two studies

	Model 1 slope (95%CI)	p-value	Model 2 slope (95%CI)	p-value	Model 3 slope (95%CI)	p-value
Men (n=2200)						
st BMI	-0.021 (-0.069, 0.026)	0.38	-0.008 (-0.055, 0.039)	0.74	-0.091 (-0.143, -0.040)	<0.001
st WHR	0.033 (-0.025, 0.090)	0.26	0.013 (-0.043, 0.069)	0.65	-0.076 (-0.136, -0.015)	0.01
Women (n=2862)						
st BMI	0.023 (-0.009, 0.056)	0.16	0.023 (-0.010, 0.055)	0.17	-0.056 (-0.091, -0.021)	0.002
st WHR	0.131 (0.093, 0.169)	<0.001	0.109 (0.070, 0.147)	<0.001	0.025 (-0.018, 0.067)	0.25

St BMI: body mass index z-score, st WHR:waist-to-hip ratio z-score

Model 1: adjusted for categorical age (5-year) and study, Model 2: adjust for variables in Model 1 plus potential confounders (smoking and education), Model 3: adjusted for variables in Model 2 plus potential mediators (systolic blood pressure, HDL cholesterol, LDL cholesterol, glycated haemoglobin, medical history of diabetes

Figure 1a: Odds ratios for having at least one plaque in KYH vs Tromsø7 with and without adjustment for adiposity

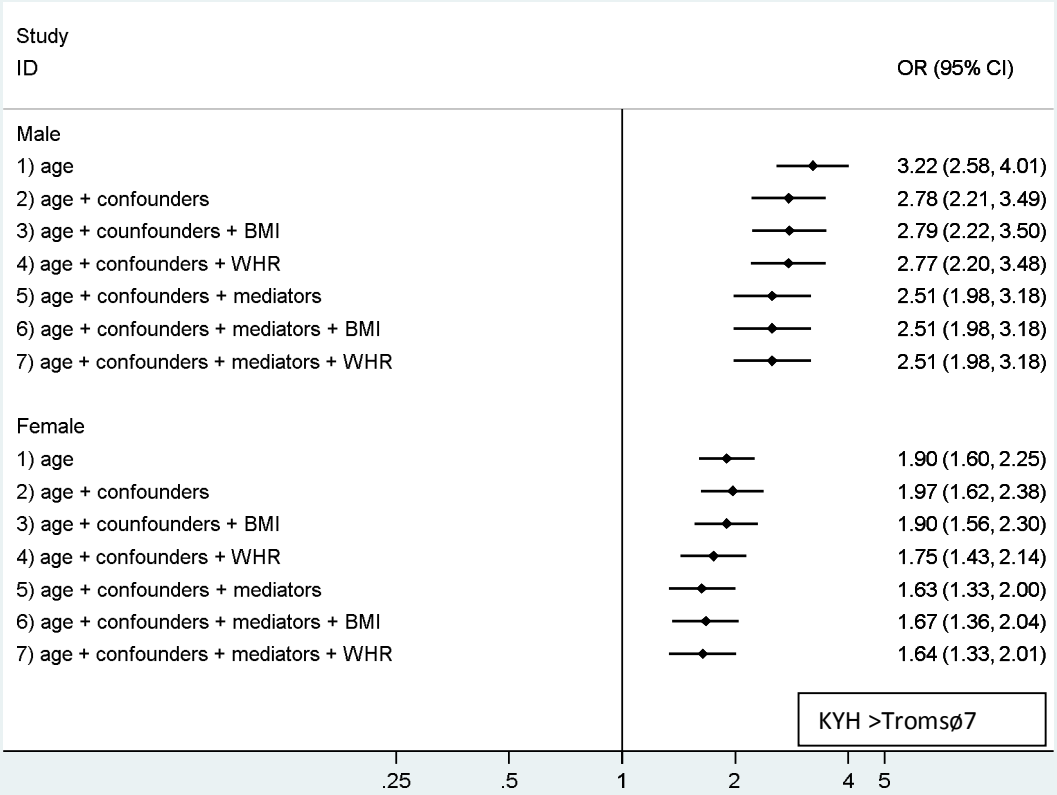
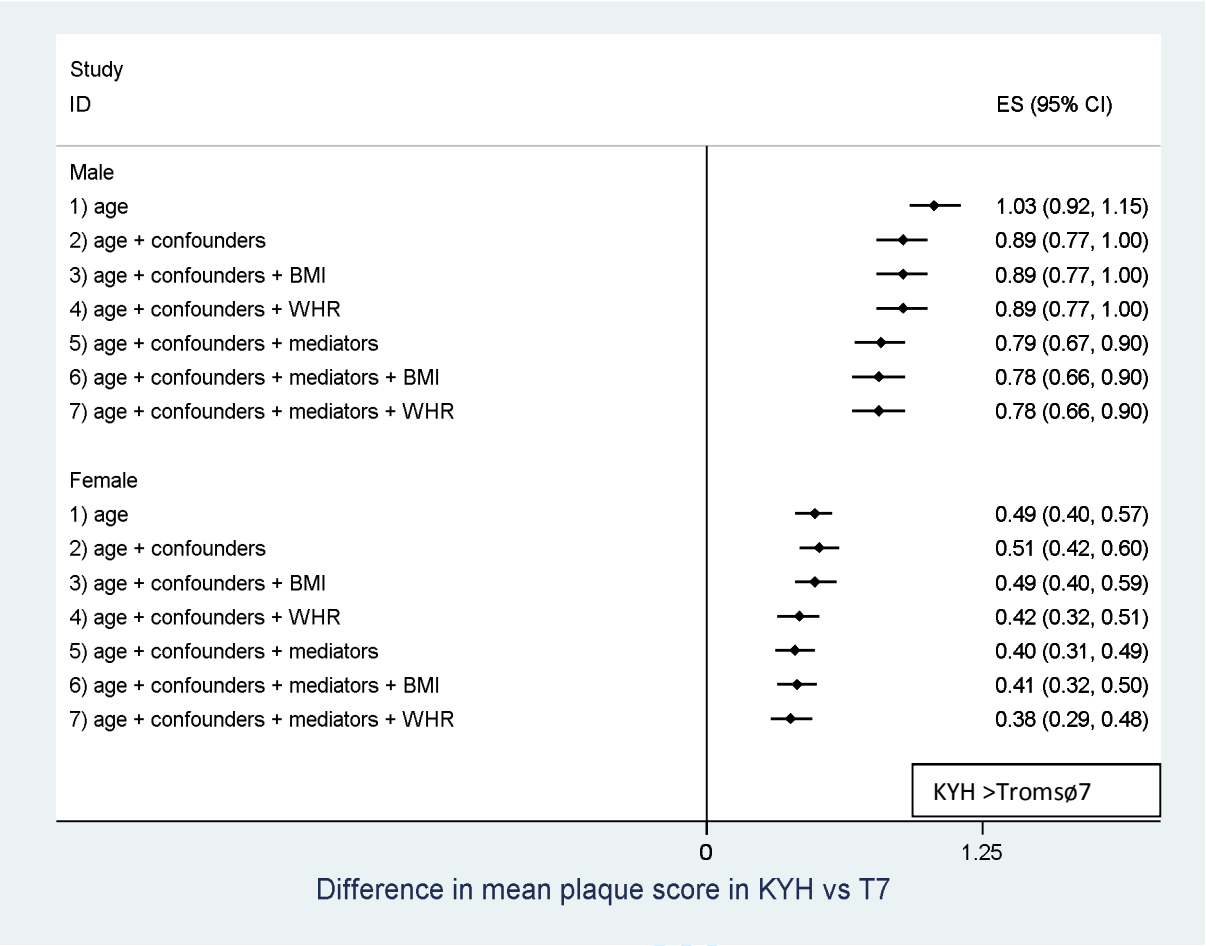


Figure 1b: Differences (95%CI) in the mean plaque score in KYH compared to Tromsø7 with and without adjustment for adiposity



Supplementary material

Title: The effect of adiposity on differences in carotid plaque burden in studies conducted in Norway and Russia: a cross-sectional analysis of two populations at very different risk of cardiovascular mortality

The name of authors

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Supplement material: the assessment of anthropometric measures

Height and weight were measured without shoes in light clothing. Height was measured to the nearest millimetre using a Seca® 217 portable stadiometer (Seca limited) in KYH and an electronic stadiometer (DS-103, Dongsahn JENIX Co. Ltd) in Tromsø7. Weight was measured to the nearest 100g with a TANITA BC 418 body composition analyser (TANITA, Europe GmbH) in KYH and an electronic digital scale (DS-B02, Dongsahn JENIX Co.Ltd) in Tromsø 7.

Supplementary table 1: Interaction

Interaction between study and adiposity

Interaction: odds ratio for having at least one plaque per 1 SD increase in each adiposity measure (adiposity#study)

men	Pooled Model 1	Pooled Model 2	Pooled Model 3
BMI	0.22	0.35	0.39
WHR	0.24	0.42	0.29
women			
BMI	0.11	0.044	0.21
WHR	0.72	0.79	0.73

Model 1: adjusted for categorical age (5-year) and study, Model 2: adjust for variables in Model 1 plus potential confounders (smoking and education), Model 3: adjusted for variables in Model 2 plus potential mediators (systolic blood pressure, HDL cholesterol, LDL cholesterol, glycated haemoglobin, diabetes)

Interaction: Change in plaque score per 1 SD increase in each adiposity measure: pooled results from two studies (adiposity#study)

men	Pooled Model 1	Pooled Model 2	Pooled Model 3
BMI	0.02	0.07	0.12
WHR	0.45	0.99	0.83
women			
BMI	0.27	0.15	0.70
WHR	0.03	0.02	0.02

Model 1: adjusted for categorical age (5-year) and study, Model 2: adjust for variables in Model 1 plus potential confounders (smoking and education), Model 3: adjusted for variables in Model 2 plus potential mediators (systolic blood pressure, HDL cholesterol, LDL cholesterol, glycated haemoglobin, diabetes)

Supplementary table 2: Comparison of carotid plaque burden in KYH compared to Tromsø7 with and without adjustment for various adiposity measure (data of figure 1)

A) Odds ratios for having at least one plaque in KYH vs Tromsø7 with and without adjustment for adiposity

	Men (n=2200)	Women (n=2862)
	OR (95%CI)	OR (95%CI)
Model 1-adiposity	3.22 (2.58, 4.01)	1.90 (1.60, 2.25)
Model 2-adiposity	2.78 (2.21, 3.49)	1.97 (1.62, 2.38)
Model 2 BMI	2.79 (2.22, 3.50)	1.90 (1.56, 2.30)
Model 2 WHR	2.77 (2.20, 3.48)	1.75 (1.43, 2.14)
Model 3 - adiposity	2.51 (1.98, 3.18)	1.63 (1.33, 2.00)
Model 3 BMI	2.51 (1.98, 3.18)	1.63 (1.36, 2.04)
Model 3 WHR	2.51 (1.98, 3.18)	1.64 1.33, 2.01)

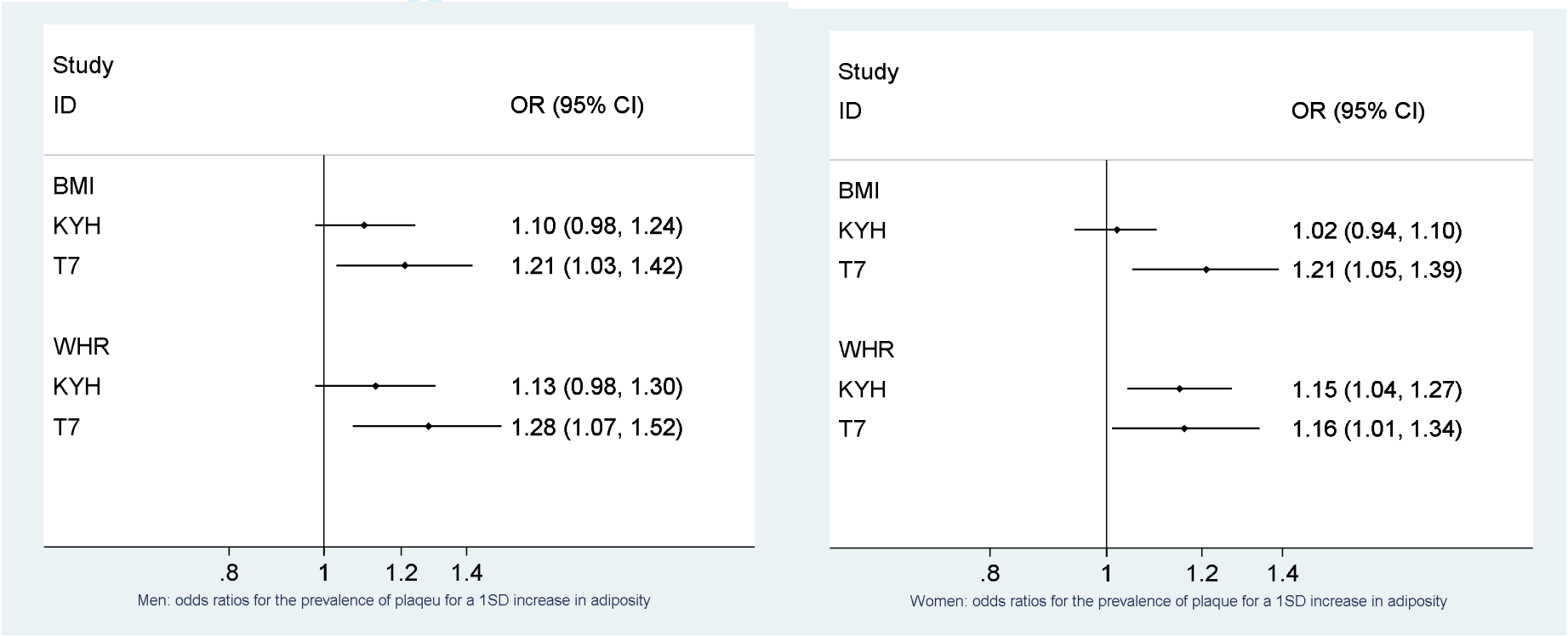
B) Differences (95%CI) in the mean number of plaques in KYH compared to Tromsø7 with and without adjustment for adiposity

	Men (n=2200)	Women (n=2862)
	Difference in number of plaque (95%CI)	Difference in number of plaque (95%CI)
Model 1-adiposity	1.03 (0.92, 1.15)	0.49 (0.40, 0.57)
Model 2-adiposity	0.89 (0.77, 1.00)	0.51 (0.42, 0.60)
Model 2 BMI	0.89 (0.77, 1.00)	0.49 (0.40, 0.59)
Model 2 WHR	0.89 (0.77, 1.00)	0.42 (0.32, 0.51)
Model 3 - adiposity	0.79 (0.67, 0.90)	0.40 (0.31, 0.49)
Model 3 BMI	0.78 (0.66, 0.90)	0.41 (0.32, 0.50)
Model 3 WHR	0.78 (0.66, 0.90)	0.38 (0.29, 0.48)

Supplementary figure

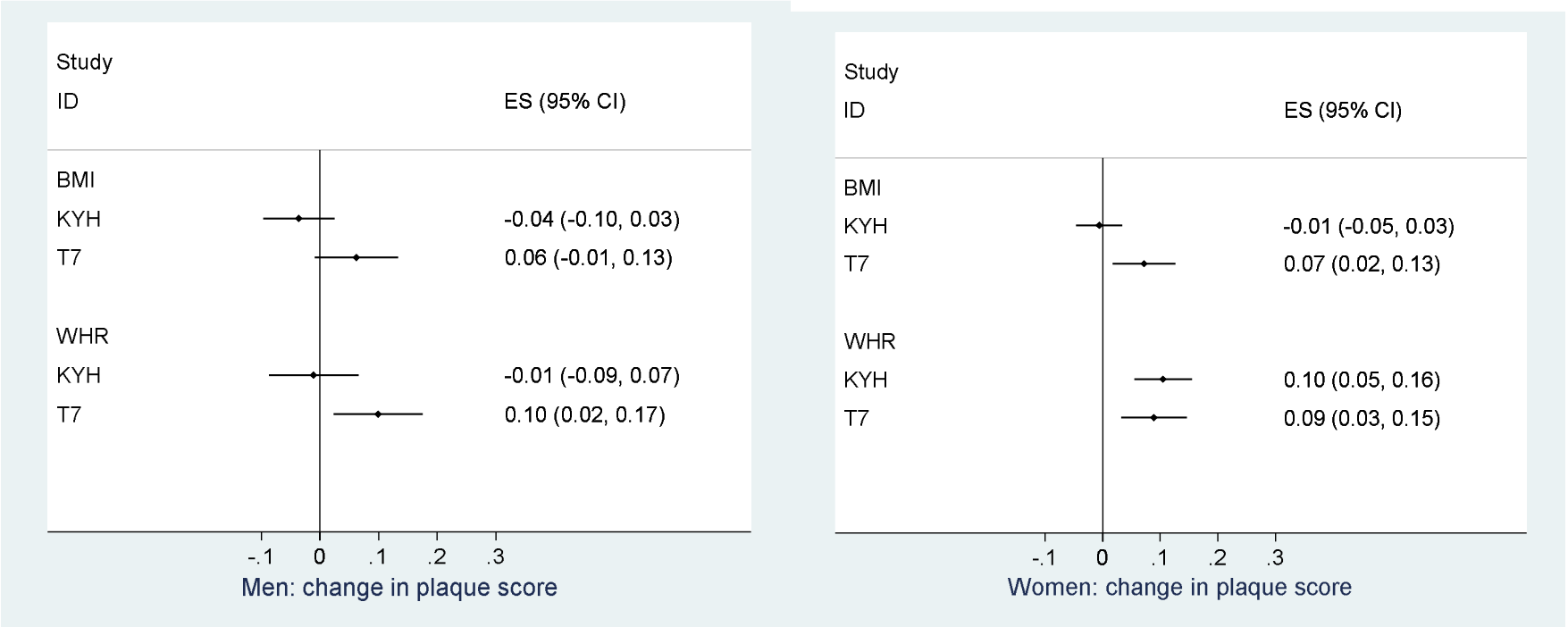
Supplementary figure 1A

Study-specific odds ratio for the prevalence of plaques for a 1 SD increase in each adiposity measure after the adjustment for age, smoking, and education (Model 2) (left: men, right: woman)



Supplementary figure 1B

Study-specific change in plaque score per 1 SD increase in each adiposity measure after the adjustment for age, smoking, and education (Model 2) (left: men, right: women)



STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5-6*
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	5
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	5
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	NA

Outcome data	15*	Report numbers of outcome events or summary measures	7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	16-17
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Supplementary
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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The effect of adiposity on differences in carotid plaque burden in studies conducted in Norway and Russia: a cross-sectional analysis of two populations at very different risk of cardiovascular mortality

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The effect of adiposity on differences in carotid plaque burden in studies conducted in Norway and Russia: a cross-sectional analysis of two populations at very different risk of cardiovascular mortality

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Key words

Epidemiology, Carotid atherosclerosis, Adiposity, Russian Federation, Europe

Word counts

3025 words

Abstract

Objectives: Large differences exist in the burden of cardiovascular disease (CVD) between Russia and Western European countries including Norway. Obesity prevalence may contribute to the differences. We investigated whether difference in the level of adiposity, assessed using body mass index (BMI) and waist-to-hip ratio(WHR), could explain inter-country differences in the burden of carotid plaque, a measure of atherosclerosis, in the populations.

Design: Cross-sectional analysis. Logistic and linear regression models were used.

Setting: We used population-based cross-sectional Know Your Heart (KYH) study in Russia and the Tromsø 7 study (Tromsø 7) in Norway.

Participants: 3262 and 1800 men and women aged 40-69 years in KYH and Tromsø 7, respectively.

Primary and secondary outcome: The presence of carotid plaques and plaque score assessed using ultrasound.

Results: The presence of carotid plaques and plaque score were higher in KYH than Tromsø 7 regardless of age group and sex. A positive association between carotid plaque burden and adiposity was found (OR of having at least one plaque per SD in WHR 1.18 (95% CI 1.06, 1.31) for men; 1.15 (1.06, 1.25) for women)) adjusted for age, smoking and education in a pooled analysis of the two studies. There was little evidence of the interaction between study and adiposity. These effects did not differ between the two studies. However, neither adiposity nor CVD risk factors (smoking, systolic blood pressure, cholesterol, glycosylated haemoglobin) explained the higher carotid plaque burden in KYH compared to Tromsø 7.

Conclusion: Adiposity, especially abdominal adiposity, is a risk factor for carotid plaque in Russia and Norway, although neither adiposity nor established CVD risk factors explained the higher plaque burden in Russia. To reduce the CVD burden in Russia, beyond prevention and treatment of adiposity, further research is required to understand why Russia has a high burden of atherosclerosis.

Strengths and limitations of this study

- This is the first study to compare adiposity level, carotid plaque burden, and its association with adiposity between Russia and Western European country with low CVD mortality.
- The use of two substantial population-based studies with similar study period and study protocols enabled us to make a direct comparison of two populations.
- Waist circumference was measured at different measurement sites between the two studies.
- We did not assess visceral adiposity or body composition.

Introduction

The mortality rate from cardiovascular disease (CVD) has been decreasing for many years in Western Europe, and more recently in Eastern Europe (1). However, rates vary substantially between countries, with Russia having one of the highest CVD mortality rates (2), although it has been declining since 2005 (3). In 2012-16, the CVD mortality rate at working-ages in Russia was eight times higher than that in Norway (4). These premature deaths contribute to the relatively low life expectancy for such an industrialised country. However, the reasons for this very high CVD burden in Russia remain unclear (4). It appears that the differences in conventional CVD risk factors such as smoking, blood pressure, and cholesterol levels do not explain this difference well (5, 6).

The increase in obesity over the past decades is a growing concern worldwide, including in Russia and countries of Western Europe (7), and has an effect on mortality levels (8). In addition to general obesity, however, the extent of abdominal obesity is likely to be important as there is evidence that it is more strongly associated with CVD events than general adiposity assessed using body mass index (BMI) (9-11). However, data on population-levels of abdominal obesity (such as waist-hip ratio (WHR)) is far less common than for BMI, in Russia as well as in other countries. To the best of our knowledge, the contribution of general or abdominal obesity to the gap in CVD burden between Russia and Western European countries has not been investigated in spite of increasing importance of obesity as a CVD risk factor.

Carotid plaque, representing an advanced stage of atherosclerosis, is predictive of future CVD events (12). Carotid atherosclerosis may be easily and reliably detected using an ultrasound examination making carotid plaque a good surrogate marker of atherosclerotic CVD burden in large-scale epidemiological studies. Our previous study using Tromsø Study fifth survey has shown that abdominal adiposity was more closely associated with carotid plaque burden than BMI (13). Furthermore, WHR showed the larger effect size than waist circumference and waist to height ratio.

We used data from two studies from general populations with very different CVD mortality in Europe: Know Your Heart (KYH) study in Russia and Tromsø Study seventh survey (Tromsø 7) in Norway. Our aims were: 1) to compare general and abdominal adiposity levels, represented by BMI and WHR, respectively, and the burden of carotid plaque in Russia with those in Norway, a low CVD mortality country; 2) to investigate the association of BMI or WHR with carotid plaque in both populations; and 3) to investigate whether BMI or WHR or other factors can explain difference in carotid plaque burden between the two populations.

Methods

Study design and participants

We used data from two studies; the Know Your Heart study (KYH) from Russia and the Tromsø 7 study from Norway. Researchers from the two studies worked together at the design stage to align aspects of the study protocols used, including the detailed standard operating procedures for carotid ultrasound examinations as described elsewhere (4, 14, 15).

KYH is a population-based cross-sectional study of 4500 women and men aged 35-69 years conducted between 2015 and 2017 in two Russian cities: Novosibirsk and Arkhangelsk. The details of KYH have been described elsewhere (4). Briefly, participants were recruited from a random sample of the population stratified by age and gender, derived from the list of the Territorial Health Insurance Funds. Trained interviewers visited the addresses on the list and identified residents of the target age and sex. Information on socio-demographic characteristics, CVD risk factors and medical history were collected using structured questionnaires completed on tablet computers. At the end of the interview, participants were invited to have a comprehensive examination including anthropometric measurement, blood sampling, and carotid ultrasound one or two weeks later. Response rates for initial interview were 53% and 27% in Arkhangelsk and Novosibirsk, respectively. Of those interviewed 89% attended the subsequent medical examination. All participants of the medical examination provided signed informed consent.

The Tromsø Study is an ongoing population-based study in Tromsø municipality, North Norway, and consists of seven surveys from 1974-2016 (16). In the seventh wave (Tromsø 7), all residents in Tromsø aged 40 years and older were invited to participate. The questionnaires were completed, a brief physical examination was carried out, and biological samples were taken. A random sample including previous participants were invited to a second visit to undergo more comprehensive medical examinations. A total of 21083 attended the first visit and the response rate was 65%. 4153 participants were invited to a carotid ultrasound examination and 2974 (71.6%) attended.

In the two studies, participants aged between 40-69 years (n=5782) were eligible for the present study. We excluded participants with missing data on all adiposity measures (n=42), and potential confounders and mediators (n=678), leaving 3262 participants from KYH (57% women) and 1800 from Tromsø 7 (55% women) for the analyses.

Assessment of anthropometric measures and other CVD risk factors

In both studies, height and weight were assessed by trained staff using standard methods (see supplementary material). BMI was calculated by dividing weight in kilograms by squared height in meters. Waist circumference (WC) was measured at a different site in the two studies: in KYH WC was measured at the narrowest part of the trunk to the nearest millimetre using a tape measure while in Tromsø 7 WC was measured at the level of the umbilicus. Hip circumference was measured at the widest part in both studies. To ensure WC was comparable between the two studies, WC in Tromsø 7 was converted to the narrowest waist using a conversion equation by Mason et al. (17). Among

anthropometric measures of abdominal adiposity WHR was selected because it has been found to be strongly associated with CVD events (10, 11, 18).

Information on age (5-year categories), smoking (current smoker, ex-smoker, never-smoker), educational attainment (higher education: Yes/No), and medical history of diabetes (DM) (Yes/No) were collected through face-to-face interview in KYH and self-administered questionnaire in Tromsø 7. The assessment of systolic blood pressure (SBP) and other laboratory data are described elsewhere (see supplementary material) (4).

Ultrasound examination

Technical details of the examination protocols have been described elsewhere (4, 14, 15). Briefly, both carotid arteries were scanned for carotid plaques in the common carotid artery (CCA), bifurcation and internal carotid artery (ICA) using a Vivid Q (GE Health care) with 6~13 MHz linear transducers in KYH and Vivid 7 (GE Health care) with a linear 12 MHz transducer in Tromsø 7 (see supplementary material). Carotid plaque was defined according to the Mannheim Consensus as a focal structure encroaching into the arterial lumen by at least 0.5 mm, or having a thickness ≥50% greater than the surrounding intima-media thickness (IMT), or IMT >1.5 mm as measured from the media-adventitia interface to the intima-lumen interface (19).

To evaluate the burden of carotid plaque, we created a cumulative plaque score by assigning a score of one for the presence of one or more plaques in each of the six carotid segments (CCA, bifurcation, and ICA of each carotid artery) with a maximum possible score of six for each individual.

Statistical methods

Analyses were conducted stratifying by sex a priori. Two outcome measures were used: the presence of plaques as a binary outcome and plaque score. As exposures, BMI and WHR were used to represent general and abdominal adiposity, respectively. To enable direct comparison of the magnitude of the effects of BMI and WHR, sex-specific adiposity z-scores standardised to Tromsø 7 participants were created by subtracting the mean and dividing by the standard deviation (SD) of each measure in Tromsø 7.

Variables included in the model were selected from established CVD risk factors (2, 20). Age, smoking, and education were considered a priori confounders while SBP, HDL cholesterol, LDL cholesterol, glycosylated haemoglobin (HbA1c) and medical history of DM were considered as potential mediators. Sex-specific linear and logistic regression models were used to investigate the associations of each adiposity with plaque score and presence of plaques respectively. A series of models were fitted that were specific to each sex and study (4 in all). Model 1 adjusted for age (5-year age-groups). Model 2, our main model to elucidate the association between adiposity and plaque burden, further adjusted for potential confounders. Model 3 further adjusted for potential mediators to

see to what extent the association was mediated by these factors. These analyses were conducted using the data from each study and the pooled data from the two studies after checking for interaction with study. This was done by adding an interaction term between study and adiposity using pooled data: testing for statistical significance using likelihood ratio tests for logistic regression and Wald tests for linear regression.

Finally, to estimate the difference in plaque burden between the two studies, we applied a similar set of models as already described to the pooled data using a binary indicator for study. The associations between each study and plaque burden (the presence of plaques, plaque score) were estimated using logistic and linear regression, respectively. To look at adjusted difference in plaque burden between the two studies, three similar models adjusted for age, confounders, and mediators, were applied without adjustment for adiposity. We then separately added each adiposity measure to these models to estimate the effect of adiposity on between-study difference in carotid plaque burden.

STATA version 15 (Stata Corp) was used for all the analyses.

Ethical approval for the KYH study was received from the ethics committees of the London School of Hygiene & Tropical Medicine, Novosibirsk State Medical University, the Institute of Internal and Preventative Medicine, Novosibirsk and the Northern State Medical University, Arkhangelsk . Ethical approval for Tromsø 7 was obtained the Regional Committee for Medical and Health Research Ethics (reference number 2014/940).

Patient and public involvement

This study was part of the International Project on Cardiovascular Disease in Russia (IPCDR). IPCDR had an important Public Engagement component as described on the project website (https://knowyourheart.science/). This involved a wide range of activities that ranged from television programmes on the Know Your Heart study, focus groups and publication of popular articles on cardiovascular disease in the Russian media. The Heart to Heart comparisons of Norway with Russia have received media coverage in the Norwegian media, and the Tromsø 7 study itself involved extensive publicity engagement with the citizens of the city of Tromsø. Members of the general public were not involved in the design or the study or its scientific aims.

Results

Baseline characteristics of participants

Table 1 shows participants' baseline characteristics. The age-adjusted prevalence of current smoking in men was much higher in KYH than Tromsø 7 but similar for women. However, female never smokers made up two-thirds in KYH but just over a third in Tromsø 7. Mean SBP was considerably higher in KYH than in Tromsø 7.

Adiposity

Both BMI and WHR were higher for women in KYH than those in Tromsø 7. Adiposity z-scores for BMI and WHR for the KYH women standardised to the Tromsø 7 population adjusted for age were 0.58 (95% CI: 0.49, 0.68) and 0.84 (95% CI: 0.76, 0.92), respectively. However, adiposity did not differ between men in the two studies.

Prevalence of carotid plaques

The prevalence of carotid plaques and the mean plaque score increased with age in both women and men (Table 2). The burden of plaques was consistently higher in KYH than Tromsø 7 in both sexes.

The association between carotid plaque burden and adiposity: a pooled analysis of the two studies

Table 3 shows the odds ratios for having at least one carotid plaque per 1SD increase in each adiposity measure by sex from the pooled analysis. We also analysed the two studies separately, but only presented the result from the pooled data based on the test of interaction described below. The two adiposity measures were not adjusted for each other. After adjustment for confounders (Model 2), there was evidence of association between all adiposity measures and the presence of plaques except for BMI in women. WHR showed larger standardized odds ratios (women 1.15 95% CI: 1.06, 1.25, men 1.18 95% CI: 1.06, 1.31) than BMI. After further adjustment for cardio-metabolic mediators (Model 3), all odds ratios decreased substantially.

Table 4 shows the difference in plaque score per 1SD increase in each adiposity measure. In women, adiposity was associated with an increase in plaque score. Again, WHR showed a larger effect size (increase per 1SD change 0.109 95% CI: 0.070, 0.147) than BMI. Additional adjustments for cardio-metabolic mediators reduced both effect sizes substantially. For men, there was no evidence of an association of BMI and WHR with plaque score.

Tests for interaction between study and adiposity were not statistically significant except for the association between BMI and the presence of plaque in women and that between WHR and the plaque score in women, suggesting that there is little evidence that the association between adiposity and plaque burden differs between the two studies (Supplementary table 1, Supplementary figure 1A, 1B).

Between-study differences in carotid plaque burden and the effect of adiposity

Figure 1 compares the carotid plaque burden between the two studies with and without adjustment for adiposity. Without adjustment for adiposity measures, the odds ratio for having at least one plaque in KYH compared to Tromsø 7 was 1.97 (95% CI: 1.62, 2.38) in women and 2.78 (95% CI: 2.21, 3.49) in men (Figure 1a, Supplementary table 2A Model 2). Further adjustment for BMI or WHR separately had only a small effect on this odds ratio for both men and women (Figure 1a). The between-study

difference remained large and statistically significant after further adjustment for cardio-metabolic mediators (Figure 1a, Supplementary table 2A Model 3).

Similarly, without adjustment for adiposity, participants in KYH had a higher mean plaque score than those in Tromsø 7 by 0.51 (95% CI: 0.42, 0.60) for women and 0.89 (95% CI: 0.77, 1.00) for men; these estimates decreased slightly for women and hardly changed at all for men with further adjustment for adiposity (Figure 1b, Supplementary table 2B). The between-study difference remained significant after further adjustment for cardio-metabolic mediators (Figure 1b, Supplementary table 2B Model 3).

Discussion

There was evidence of positive associations between adiposity, especially abdominal adiposity, and carotid plaque burden, but no convincing evidence that the strength of these associations differed between the two studies. These associations were largely mediated by cardio-metabolic CVD risk factors. However, neither adiposity nor the confounders and potential mediators explained the substantially greater burden of plaque in the KYH study in Russia compared to the Tromsø 7 study in Norway. To the best of our knowledge, this is the first study to directly investigate the role of adiposity in high CVD burden in a general population in Russia in comparison with another country.

Given the similar adiposity level between KYH and Tromsø 7 among men, it is not surprising that higher plaque burden in men in KYH is not explained by adiposity. However, even among women whose adiposity level was considerably higher in KYH than Tromsø 7, the adjustment for adiposity had little impact on the inter-study difference in carotid plaque burden. Furthermore, additional adjustment for CVD and metabolic risk factors such as smoking, systolic blood pressure and cholesterol level slightly reduced this inter-study difference, but the between-study difference remained for both men and women, suggesting that there are other determinants of higher carotid plaque burden in a population in KYH. This is consistent with previous studies conducted 20 or more years ago, showing that differences in traditional CVD risk factors did not fully explain the high CVD burden in Russia compared to Western European countries (5, 6). More advanced subclinical atherosclerosis in participants in KYH compared to Tromsø 7 is in keeping with the higher CVD mortality rate in Russia than Western European countries (4, 21).

The development of coronary artery disease and atherosclerotic plaque is a gradual process that occurs across the lifecourse (22). The extent to which single cross-sectional measurements of risk factors such as blood pressure and smoking can capture the full impact of these risk factors on the burden of carotid plaque is therefore questionable. In making the sort of comparisons between populations that are the focus of this paper therefore, it could be that we are underestimating the potential contribution of these risk factors to differences in plaque burden, particularly if risk factor profiles have been changing.

One potential determinant of high CVD risk in Russia that we have not included is alcohol which has been shown to be related to mortality from cardiovascular disease(23). Vikhireva et al. added hazardous alcohol consumption to the high-risk version of SCORE to see whether this modified model improved prognostic performance of SCORE for future CVD events in the Russian population. However, this modification did not improve the prediction of CVD events (24) although the study had limited follow-up and relatively small numbers of events. Moreover, it excluded as an outcome alcoholic cardiomyopathy that contributes to the high CVD mortality in Russia (25) involving processes other than atherosclerosis (26). Differences in treatment and access to the medical facilities between Western Europe and Russia is likely to partly account for the higher CVD mortality rates in Russia, but it is unlikely that differences in treatment could account for the differences in subclinical atherosclerosis in a population-based samples. Furthermore, the treatment and access to appropriate medical care have been improving rapidly in Russia, especially in large cities, so this is likely to be a less important factor in the future (27, 28). Identification of the determinant(s) of advanced subclinical atherosclerosis in Russia will be important to target interventions to reduce CVD burden.

There was evidence of positive associations between adiposity and plaque burden in both studies emphasising the importance of the control of adiposity, especially abdominal adiposity, to curb the CVD burden in both countries. The prevalence of obesity in Russia has been increasing (7), with the notably high level among women being of particular concern (7). Another important implication of our findings is the importance of the control of cardiovascular and cardio-metabolic mediators. The associations between adiposity and carotid plaque burden were largely mediated by SBP, cholesterol level and HbA1c. The effective control of these traditional risk factors will mitigate the negative effect of adiposity.

This study is the first, to our knowledge, to bring together data from a high and relatively low CVD mortality country to investigate the association of adiposity with carotid plaque, and also the extent to which this can explain differences in the burden of carotid plaque between the two populations. Moreover, this was done using ultrasound examination protocols that were aligned between the two studies. No previous studies have compared imaging of atherosclerotic changes in general populations between Russia and Western countries. However, our investigation has some limitations. First, the anthropometric measures we used are crude measures of visceral adiposity. However, estimation of visceral adipose tissue using MRI and CT is resource-demanding and logistically difficult in large epidemiological studies. Second, WC was measured differently between the two studies. Although the conversion of WC was made using a conversion equation, this did not allow for individual variability. Standardisation of the protocol of WC measurement would be important in future studies. Third, we did not include alcohol in our regression models, although it is likely to play an important role in CVD mortality in Russia (29-32). This is because alcohol consumption in the two study populations were not directly comparable. Finally, as always, caution must be exercised in generalising to the

national situation the results we have obtained from the two cross-sectional studies of selected groups in two cities in Russian and one in Norway city.

Overall, our findings have two implications with respect to tackling the high CVD burden in Russia. First, although adiposity failed to explain higher plaque burden in Russia compared to Norway, adiposity, especially abdominal adiposity, appeared to contribute to an increase in carotid plaque burden through cardio-metabolic mediators such as blood pressure and cholesterol. The reduction of adiposity level will be important to avoid further CVD burden in addition to the control of cardio-metabolic mediators. Second, our findings suggest that there are other unidentified risk factors that determine the higher carotid plaque burden in Russia compared to Norway. Further studies will be needed to identify them.

References

1. Ezzati M, Obermeyer Z, Tzoulaki I, Mayosi BM, Elliott P, Leon DA. Contributions of risk factors and medical care to cardiovascular mortality trends. Nature reviews Cardiology. 2015;12(9):508-30.
2. Timmis A, Townsend N, Gale C, Grobbee R, Maniadakis N, Flather M, et al. European Society of Cardiology: Cardiovascular Disease Statistics 2017. Eur Heart J. 2018;39(7):508-79.
3. Grigoriev P, Meslé F, Shkolnikov VM, Andreev E, Fihel A, Pechholdova M, et al. The recent mortality decline in Russia: Beginning of the cardiovascular revolution? 2014;40(1):107-29.
4. Cook S, Malyutina S, Kudryavtsev A, Averina M, Bobrova N, Boytsov S, et al. Know Your Heart: Rationale, design and conduct of a cross-sectional study of cardiovascular structure, function and risk factors in 4500 men and women aged 35-69 years from two Russian cities, 2015-18 [version 1; referees: 1 approved, 1 approved with reservations]. Wellcome Open Research. 2018;3(67).
5. Kuulasmaa K, Tunstall-Pedoe H, Dobson A, Fortmann S, Sans S, Tolonen H, et al. Estimation of contribution of changes in classic risk factors to trends in coronary-event rates across the WHO MONICA Project populations. Lancet. 2000;355(9205):675-87.
6. Averina M, Nilssen O, Brenn T, Brox J, Kalinin AG, Arkhipovsky VL. High cardiovascular mortality in Russia cannot be explained by the classical risk factors. The Arkhangelsk Study 2000. European journal of epidemiology. 2003;18(9):871-8.
7. NCD Risk Factor Collaboration. Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. Lancet (London, England). 2016;387(10026):1377-96.
8. Vidra N, Trias-Llimos S, Janssen F. Impact of obesity on life expectancy among different European countries: secondary analysis of population-level data over the 1975-2012 period. BMJ Open. 2019;9(7):e028086.
9. Peters SAE, Bots SH, Woodward M. Sex Differences in the Association Between Measures of General and Central Adiposity and the Risk of Myocardial Infarction: Results From the UK Biobank. Journal of the American Heart Association. 2018;7(5).
10. Emdin CA, Khera AV, Natarajan P, Klarin D, Zekavat SM, Hsiao AJ, et al. Genetic Association of Waist-to-Hip Ratio With Cardiometabolic Traits, Type 2 Diabetes, and Coronary Heart Disease. Jama. 2017;317(6):626-34.
11. Dale CE, Fatemifar G, Palmer TM, White J, Prieto-Merino D, Zabaneh D, et al. Causal Associations of Adiposity and Body Fat Distribution With Coronary Heart Disease, Stroke Subtypes, and Type 2 Diabetes Mellitus: A Mendelian Randomization Analysis. Circulation. 2017;135(24):2373-88.
12. Inaba Y, Chen JA, Bergmann SR. Carotid plaque, compared with carotid intima-media thickness, more accurately predicts coronary artery disease events: a meta-analysis. Atherosclerosis. 2012;220(1):128-33.
13. Imahori Y, Mathiesen EB, Leon DA, Hopstock LA, Hughes AD, Johnsen SH, et al. The contribution of obesity to carotid atherosclerotic plaque burden in a general population sample in Norway: The Tromso Study. Atherosclerosis. 2018;273:15-20.
14. Fosse E, Johnsen SH, Stensland-Bugge E, Joakimsen O, Mathiesen EB, Arnesen E, et al. Repeated visual and computer-assisted carotid plaque characterization in a longitudinal population-based ultrasound study: the Tromso study. Ultrasound in medicine & biology. 2006;32(1):3-11.
15. Imahori Y. Adiposity and carotid atherosclerosis in two populations at very different risk of cardiovascular mortality: Norway and Russia [dissertation on the Internet]. London School of Hygiene & Tropical Medicine; 2019. Available from: https://researchonline.lshtm.ac.uk/id/eprint/4654524/
16. Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njolstad I. Cohort profile: the Tromso Study. International journal of epidemiology. 2012;41(4):961-7.

17. Mason C, Katzmarzyk PT. Variability in waist circumference measurements according to anatomic measurement site. Obesity (Silver Spring). 2009;17(9):1789-95.
18. O'Donnell MJ, Chin SL, Rangarajan S, Xavier D, Liu L, Zhang H, et al. Global and regional effects of potentially modifiable risk factors associated with acute stroke in 32 countries (INTERSTROKE): a case-control study. Lancet (London, England). 2016;388(10046):761-75.
19. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, et al. Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011. Cerebrovascular diseases (Basel, Switzerland). 2012;34(4):290-6.
20. Lloyd-Jones DM, Hong Y, Labarthe D, Mozaffarian D, Appel LJ, Van Horn L, et al. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. Circulation. 2010;121(4):586-613.
21. Townsend N, Wilson L, Bhatnagar P, Wickramasinghe K, Rayner M, Nichols M. Cardiovascular disease in Europe: epidemiological update 2016. European heart journal. 2016.
22. Rose G. Incubation period of coronary heart disease. British medical journal (Clinical research ed). 1982;284(6329):1600-1.
23. Leon DA, Shkolnikov VM, McKee M, Kiryanov N, Andreev E. Alcohol increases circulatory disease mortality in Russia: acute and chronic effects or misattribution of cause? International journal of epidemiology. 2010;39(5):1279-90.
24. Vikhireva O, Kubinova R, Malyutina S, Pajak A, Simonova G, Bobak M, et al. Inclusion of hazardous drinking does not improve the SCORE performance in men from Central and Eastern Europe: the findings from the HAPIEE cohorts. BMC Public Health. 2014;14:1187.
25. Manthey J, Probst C, Rylett M, Rehm J. National, regional and global mortality due to alcoholic cardiomyopathy in 2015. Heart. 2018;104(20):1663-9.
26. Leon DA, Shkolnikov VM, Borinskaya S, Casas JP, Evans A, Gil A, et al. Hazardous alcohol consumption is associated with increased levels of B-type natriuretic peptide: evidence from two population-based studies. European journal of epidemiology. 2013;28(5):393-404.
27. Timonin S, Kontsevaya A, McKee M, Leon DA. Reducing geographic inequalities in access times for acute treatment of myocardial infarction in a large country: the example of Russia. Int J Epidemiol. 2018;47(5):1594-602.
28. Kontsevaya A, Sabgaida T, Ivanova A, Leon DA, McKee M. How has the management of acute coronary syndrome changed in the Russian Federation during the last 10 years? Health Policy. 2017;121(12):1274-9.
29. The burden of disease in Russia from 1980 to 2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet (London, England). 2018;392(10153):1138-46.
30. Feigin VL, Roth GA, Naghavi M, Parmar P, Krishnamurthi R, Chugh S, et al. Global burden of stroke and risk factors in 188 countries, during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. The Lancet Neurology. 2016;15(9):913-24.
31. Zaridze D, Brennan P, Boreham J, Boroda A, Karpov R, Lazarev A, et al. Alcohol and cause-specific mortality in Russia: a retrospective case-control study of 48,557 adult deaths. Lancet. 2009;373(9682):2201-14.
32. Leon DA, Saburova L, Tomkins S, Andreev E, Kiryanov N, McKee M, et al. Hazardous alcohol drinking and premature mortality in Russia: a population based case-control study. Lancet (London, England). 2007;369(9578):2001-9.

Footnotes

Contributors: YI, CF, DAL made substantial contributions to the conception and design of the work. EBM, LAH, AR, AK, MK, SM were centrally involved in the design of the Know Your Heart and/or the conduct of the associated fieldwork. YI undertook the analysis of the data with the regular input of CF, ADH and DAL.YI wrote the first drafts of the manuscript (including the interpretation of the results) which was then refined over successive versions by YI with the input of all authors. All authors approved the submitted version

All authors agreed both to be personally accountable for their own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, were appropriately investigated, resolved, and the resolution documented in the paper.

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Patient consent for publication: Not required

Ethics approval: Ethical approval for the KYH study was received from the ethics committees of the London School of Hygiene & Tropical Medicine (approval number 8808 received 24/02/2015); Novosibirsk State Medical University (approval number 75 received 21/05/2015); the Institute of Internal and Preventative Medicine (approval received 26/12/2014), Novosibirsk and the Northern State Medical University, Arkhangelsk (approval number 01/01-15 received 27/01/2015). Ethical approval for Tromsø 7 was obtained the Regional Committee for Medical and Health Research Ethics (reference number 2014/940).

Provenance and peer review: Not commissioned; externally peer reviewed

Data availability statement: Metadata is available for the Tromsø study at http://tromsoundersokelsen.uit.no/tromso/ and for the Know Your Heart study at https://metadata.knowyourheart.science/. The data used in these analyses cannot be placed in the public domain because of ethical and data protection restrictions.

Table 1: Participant characteristics in Know Your Heart (KYH) and Tromsø 7 (T7)*

	Men			Women		
	KYH	**Tromsø 7**	**Comparison† KYH vs T7**	**KYH**	**Tromsø 7**	**Comparison† KYH vs T7**
N	1389	811		1873	989	
Age years	56 (48-63)	61 (52-66)		55 (48-63)	60 (52-65)	
Anthropometric measure			**Difference (95% CI)**			**Difference (95% CI)**
Height cm	174.7 (6.6)	177.9 (6.7)	-3.9 (-4.4, -3.3)	161.1 (6.3)	164.7 (6.3)	-4.1 (-4.6, -3.6)
Weight kg	84.5 (15.5)	87.5 (12.9)	-3.6 (-4.9, -2.3)	74.5 (16.1)	71.4 (12.9)	3.4 (2.3, 4.6)
BMI kg/m^2	27.6 (4.6)	27.7 (3.7)	0.0 (-0.3, 0.4)	28.8 (6.2)	26.4 (4.7)	2.7 (2.3, 3.1)
WHR[a]	0.95 (0.07)	0.95 (0.07)	-0.00 (-0.00, 0.01)	0.84 (0.08)	0.79 (0.07)	0.06 (0.05, 0.06)
Potential confounders			**Odds Ratio (95% CI)**			**Odds Ratio (95% CI)**
Never-smoker (%)	351 (25.3)	316 (39.0)	(ref)	1304 (69.6)	383 (38.7)	(ref)
Ex-smoker (%)	518 (37.3)	377 (46.5)	1.3 (1.1, 1.7)	285 (15.2)	451 (45.6)	0.2 (0.1, 0.2)
Current smoker (%)	520 (37.4)	118 (14.6)	4.0 (3.1, 5.1)	284 (15.2)	155 (15.7)	0.5 (0.4, 0.6)
Higher education (%)	478 (34.3)	388 (47.8)	0.6 (0.5, 0.7)	701 (37.4)	495 (50.1)	0.5 (0.4, 0.6)
Potential mediators			**Difference (95% CI)**			**Difference (95% CI)**
SBP mmHg	138.6 (19.9)	132.8 (17.9)	7.3 (5.6, 8.9)	129.8(19.6)	126.4(19.4)	5.7 (4.3, 7.2)
Total cholesterol mmol/l	5.38 (1.13)	5.42 (1.05)	-0.06 (-0.16, 0.04)	5.68 (1.17)	5.62 (1.04)	0.13 (0.04, 0.21)
Triglycerides mmol/l	1.35 (0.95, -1.92)	1.50 (1.00, -2.10)	-0.05 (-0.15, 0.06)	1.23 (0.89,-1.77)	1.10 (0.80, -.60)	0.18 (0.11, 0.26)
HDL cholesterol mmol/l	1.32 (0.33)	1.41 (0.39)	-0.09 (-0.12, -0.06)	1.55 (0.36)	1.78 (0.49)	-0.24 (-0.27, -0.20)
LDL cholesterol mmol/l	3.66 (0.90)	3.63 (0.99)	0.01 (-0.07, 0.09)	3.80 (0.95)	3.60 (0.96)	0.27 (0.19, 0.34)
HbA1c (%)	5.60 (0.84)	5.74 (0.57)	-0.08 (-0.14, -0.01)	5.57 (7.69)	5.67 (0.51)	-0.04 (-0.09, 0.01)
			Odds Ratio (95%CI)			**Odds Ratio (95%CI)**
Diabetes mellitus (%)	93 (6.7)	47 (5.8)	1.4 (1.0, 2.1)	182 (9.7)	52 (5.3)	2.5 (1.8, 3.5)

Data are presented as percentages for binary variables and as mean values (SD) continuous variables, except for age, triglycerides which are presented as median (inter-quintile range (IQR)).

*Analyses were restricted to participants aged between 40 and 69 years with information on all covariates.

†all comparisons age-adjusted

[a]WC in Tromsø 7 assessed at the level of the umbilicus was converted to the narrowest WC so that it can be comparable with WC in KYH. WHR in T7 are calculated using converted WC.

BMI: body mass index, HDL: high-density lipoprotein, LDL: low-density lipoprotein, WC: waist circumference, WHR: waist-to-hip ratio

Table 2. The prevalence of carotid plaques and plaque score according to study and sex

	Men			Women		
	KYH	**Tromsø 7**	**Comparison KYH vs T7[a]**	**KYH**	**Tromsø 7**	**Comparison KYH vs T7[a]**
N	1389	811		1873	989	
Prevalence n (%)			**Odds Ratio (95% CI)**			**Odds Ratio (95% CI)**
All age	1050 (75.6)	499 (61.5)	3.2 (2.6, 4.0)	1043 (55.7)	478 (48.3)	1.9 (1.6, 2.3)
40-49	212/394 (53.8)	38/148 (25.7)	3.5 (2.3, 5.4)	174/570 (30.5)	40/189 (21.2)	1.7 (1.1, 2.5)
50-59	340/450 (75.6)	104/191 (54.5)	2.7 (1.9, 4.0)	328/605 (54.2)	112/273 (41.0)	1.7 (1.3, 2.3)
60-69	498/545 (91.4)	357/472 (75.6)	3.5 (2.4, 5.1)	541/698 (77.5)	326/527 (61.9)	2.1 (1.7, 2.7)
Plaque score mean (SD)			**Difference (95% CI)**			**Difference (95% CI)**
All age	1.9 (1.6)	1.2 (1.1)	1.0 (0.9, 1.2)	1.1 (1.3)	0.8 (0.9)	0.5 (0.4, 0.6)
40-49	1.0 (1.2)	0.3 (0.6)	0.6 (0.5, 0.8)	0.5 (0.8)	0.3 (0.6)	0.2 (0.1, 0.3)
50-59	1.8 (1.5)	0.9 (1.0)	0.9 (0.7, 1.2)	1.0 (1.2)	0.6 (0.8)	0.4 (0.3, 0.6)
60-69	2.7 (1.6)	1.5 (1.2)	1.2 (1.1, 1.4)	1.7 (1.4)	1.1 (1.0)	0.7 (0.5, 0.8)

[a]Adjusted for categorical age (5-year interval)

Table 3: Odds ratios for having at least one plaque per 1 SD increase in each adiposity measure: pooled results from the two studies

	Model 1 OR (95% CI)	p-value	Model 2 OR (95% CI)	p-value	Model 3 OR (95% CI)	p-value
Men (n=2200)						
st BMI	1.12 (1.02, 1.23)	0.02	1.13 (1.03, 1.24)	0.01	1.01 (0.91, 1.12)	0.83
st WHR	1.21 (1.08, 1.34)	0.001	1.18 (1.06, 1.31)	0.003	1.05 (0.93, 1.18)	0.46
Women (n=2862)						
st BMI	1.06 (0.99, 1.14)	0.08	1.06 (0.99, 1.13)	0.09	0.94 (0.87, 1.01)	0.08
st WHR	1.20 (1.11, 1.30)	<0.001	1.15 (1.06, 1.25)	0.001	1.00 (0.91, 1.09)	0.94

St BMI: body mass index z-score, st WHR: waist-to-hip ratio z-score, OR: odds ratio, 95%CI: 95% confidence interval

Model 1: adjusted for categorical age (5-year) and study, Model 2: adjust for variables in Model 1 plus potential confounders (smoking and education), Model 3: adjusted for variables in Model 2 plus potential mediators (systolic blood pressure, HDL cholesterol, LDL cholesterol, glycated haemoglobin, medical history of diabetes)

Table 4: Difference in plaque score per 1 SD increase in each adiposity measure: pooled results from the two studies

	Model 1 slope (95%CI)	p-value	Model 2 slope (95%CI)	p-value	Model 3 slope (95%CI)	p-value
Men (n=2200)						
st BMI	-0.021 (-0.069, 0.026)	0.38	-0.008 (-0.055, 0.039)	0.74	-0.091 (-0.143, -0.040)	<0.001
st WHR	0.033 (-0.025, 0.090)	0.26	0.013 (-0.043, 0.069)	0.65	-0.076 (-0.136, -0.015)	0.01
Women (n=2862)						
st BMI	0.023 (-0.009, 0.056)	0.16	0.023 (-0.010, 0.055)	0.17	-0.056 (-0.091, -0.021)	0.002
st WHR	0.131 (0.093, 0.169)	<0.001	0.109 (0.070, 0.147)	<0.001	0.025 (-0.018, 0.067)	0.25

St BMI: body mass index z-score, st WHR: waist-to-hip ratio z-score

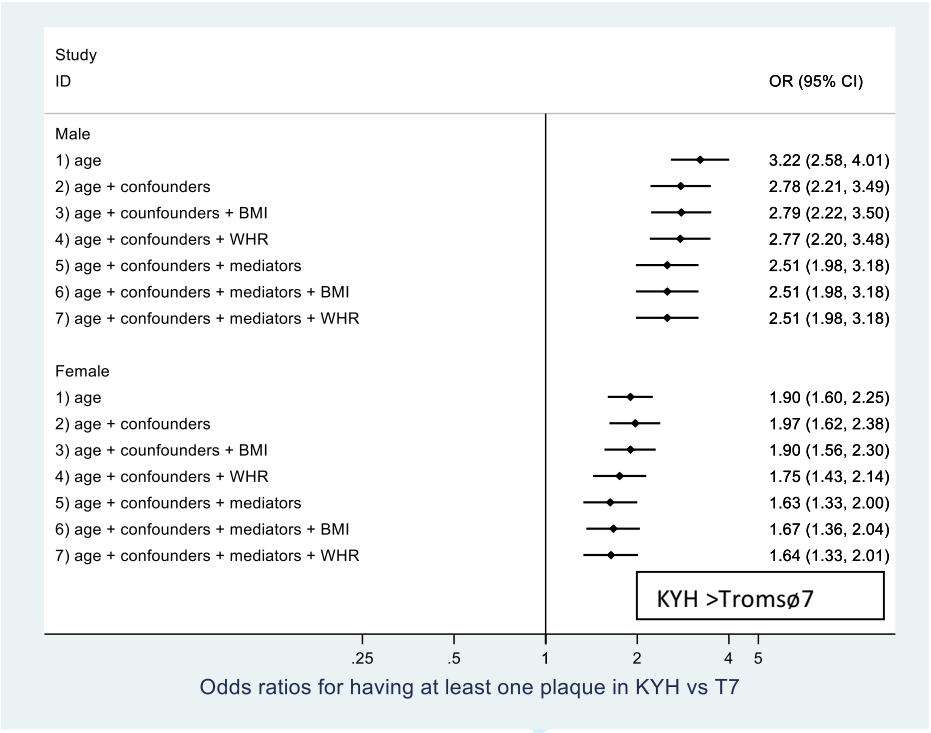
Model 1: adjusted for categorical age (5-year) and study, Model 2: adjust for variables in Model 1 plus potential confounders (smoking and education), Model 3: adjusted for variables in Model 2 plus potential mediators (systolic blood pressure, HDL cholesterol, LDL cholesterol, glycated haemoglobin, medical history of diabetes

Figure 1a: Odds ratios for having at least one plaque in KYH vs Tromsø7 with and without adjustment for adiposity

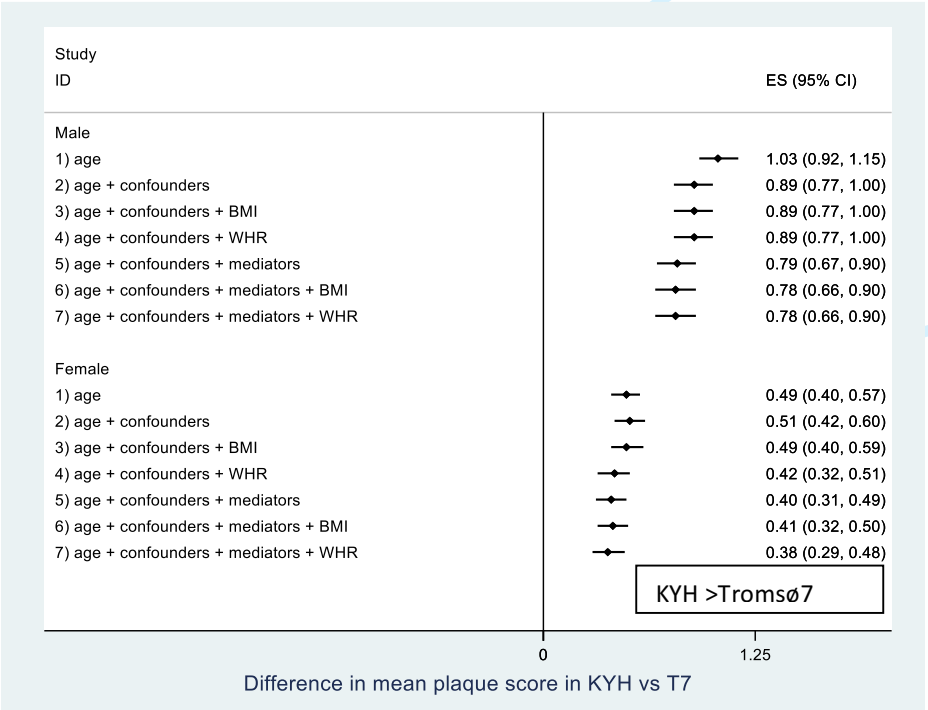
Figure 1b: Differences (95%CI) in the mean plaque score in KYH compared to Tromsø7 with and without adjustment for adiposity

Figure 1: Between-study difference in carotid plaque burden

a) Odds ratios (95%CI) for having at least one plaque in KYH vs Tromsø7 with and without adjustment for adiposity



b) Differences (95%CI) in the mean plaque score in KYH compared to Tromsø7 with and without adjustment for adiposity



BMI: body mass index, WHR: waist-to-hip ration, confounders: smoking and education, mediators: systolic blood pressure, HDL cholesterol, LDL cholesterol, glycated haemoglobin, medical history of diabetes

Supplementary material

Title: The effect of adiposity on differences in carotid plaque burden in studies conducted in Norway and Russia: a cross-sectional analysis of two populations at very different risk of cardiovascular mortality

The name of authors

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Supplementary material: the assessment of anthropometric measures

Height and weight were measured without shoes in light clothing. Height was measured to the nearest millimetre using a Seca® 217 portable stadiometer (Seca limited) in KYH and an electronic stadiometer (DS-103, Dongsahn JENIX Co. Ltd) in Tromsø7. Weight was measured to the nearest 100g with a TANITA BC 418 body composition analyser (TANITA, Europe GmbH) in KYH and an electronic digital scale (DS-B02, Dongsahn JENIX Co.Ltd) in Tromsø 7.

Ultrasound examination and the assessment of carotid plaaues

Carotid ultrasound examination was performed with the participant in a supine position by experienced sonographers in KYH and Tromsø7. In Tromsø7, the longitudinal still image of every plaque was digitally documented with the transducer pallarel to the vessel wall and perpendicular to the point of maximum plaque thickness using DICOM files for the offline reading of total plaque score. Only one plaque could be counted at each carotid segment (far and near wall of common carotid artery, bifurcation, and internal carotid artery of both carotid arteries). This means each participant could contribute to the maximum plaque number of twelve).

In KYH, off-line readings were made by two experienced cardiologists (AR and SM) to determine the presence of plaques and the actual number of plaques based on cine-loop of the carotid artery and still images of plaques using EchoPAC software (v.113, GE-Vingmed AS, Norten, Norway). The protocol of KYH did not involve recording the near or far wall location, and there was no restriction on how many plaques could be counted for each segment.

To make the burden of carotid plaques comparable between the two studies, we created a cumulative plaque score by assigning a score of one for the presence of one or more plaques in each of the six carotid segments (CCA, bifurcation, and ICA of each carotid artery) with a maximum possible score of six for each individual.

Systolic blood pressure

In KYH, SBP was measured three times, seated, using OMRON 705 IT automatic blood pressure monitors (OMRON Healthcare). Non-fasting venous blood samples were frozen within 2 hours of collection and stored at -20 degrees. Within three weeks, they were transferred to -80-degree freezers and eventually shipped to the laboratory in Moscow where all samples were analysed based on a standardised method.

In the Tromsø Study, SBP was recorded three times with Dinamap (ProCare 300, GE Healthcare). Both Dinamap and OMRON (used in KYH) have been validated to British Hypertension Society standards. Non-fasting venous blood samples were obtained, and fresh serum was analysed at the University Hospital laboratories.

Supplementary table 1: Interaction

Interaction between study and adiposity

Interaction: odds ratio for having at least one plaque per 1 SD increase in each adiposity measure (adiposity#study)

men	Pooled Model 1	Pooled Model 2	Pooled Model 3
BMI	0.22	0.35	0.39
WHR	0.24	0.42	0.29
women			
BMI	0.11	0.044	0.21
WHR	0.72	0.79	0.73

Model 1: adjusted for categorical age (5-year) and study, Model 2: adjust for variables in Model 1 plus potential confounders (smoking and education), Model 3: adjusted for variables in Model 2 plus potential mediators (systolic blood pressure, HDL cholesterol, LDL cholesterol, glycated haemoglobin, diabetes)

Interaction: Change in plaque score per 1 SD increase in each adiposity measure: pooled results from two studies (adiposity#study)

men	Pooled Model 1	Pooled Model 2	Pooled Model 3
BMI	0.02	0.07	0.12
WHR	0.45	0.99	0.83
women			
BMI	0.27	0.15	0.70
WHR	0.03	0.02	0.02

Model 1: adjusted for categorical age (5-year) and study, Model 2: adjust for variables in Model 1 plus potential confounders (smoking and education), Model 3: adjusted for variables in Model 2 plus potential mediators (systolic blood pressure, HDL cholesterol, LDL cholesterol, glycated haemoglobin, diabetes)

Supplementary table 2: Comparison of carotid plaque burden in KYH compared to Tromsø7 with and without adjustment for various adiposity measure (data of figure 1)

A) Odds ratios for having at least one plaque in KYH vs Tromsø7 with and without adjustment for adiposity

	Men (n=2200)	Women (n=2862)
	OR (95%CI)	OR (95%CI)
Model 1-adiposity	3.22 (2.58, 4.01)	1.90 (1.60, 2.25)
Model 2-adiposity	2.78 (2.21, 3.49)	1.97 (1.62, 2.38)
Model 2 BMI	2.79 (2.22, 3.50)	1.90 (1.56, 2.30)
Model 2 WHR	2.77 (2.20, 3.48)	1.75 (1.43, 2.14)
Model 3 - adiposity	2.51 (1.98, 3.18)	1.63 (1.33, 2.00)
Model 3 BMI	2.51 (1.98, 3.18)	1.63 (1.36, 2.04)
Model 3 WHR	2.51 (1.98, 3.18)	1.64 1.33, 2.01)

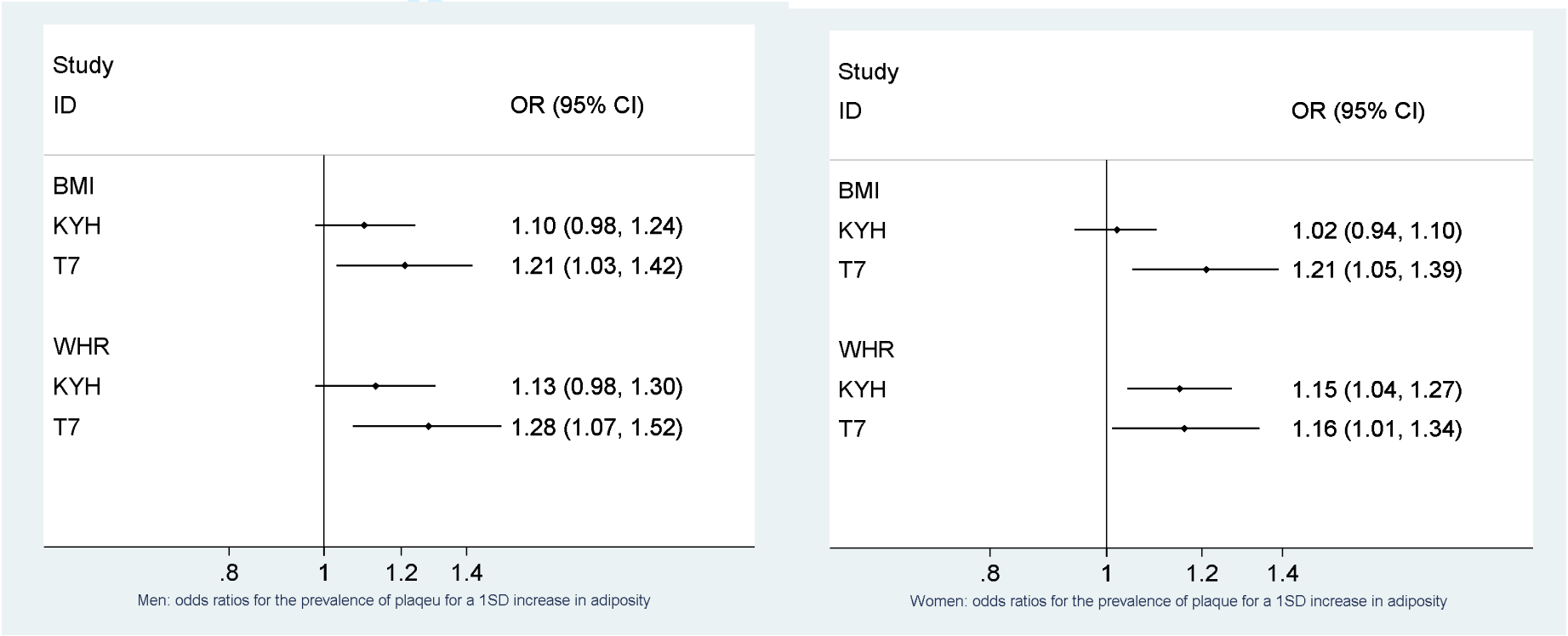
B) Differences (95%CI) in the mean number of plaques in KYH compared to Tromsø7 with and without adjustment for adiposity

	Men (n=2200)	Women (n=2862)
	Difference in number of plaque (95%CI)	Difference in number of plaque (95%CI)
Model 1-adiposity	1.03 (0.92, 1.15)	0.49 (0.40, 0.57)
Model 2-adiposity	0.89 (0.77, 1.00)	0.51 (0.42, 0.60)
Model 2 BMI	0.89 (0.77, 1.00)	0.49 (0.40, 0.59)
Model 2 WHR	0.89 (0.77, 1.00)	0.42 (0.32, 0.51)
Model 3 - adiposity	0.79 (0.67, 0.90)	0.40 (0.31, 0.49)
Model 3 BMI	0.78 (0.66, 0.90)	0.41 (0.32, 0.50)
Model 3 WHR	0.78 (0.66, 0.90)	0.38 (0.29, 0.48)

Supplementary figure

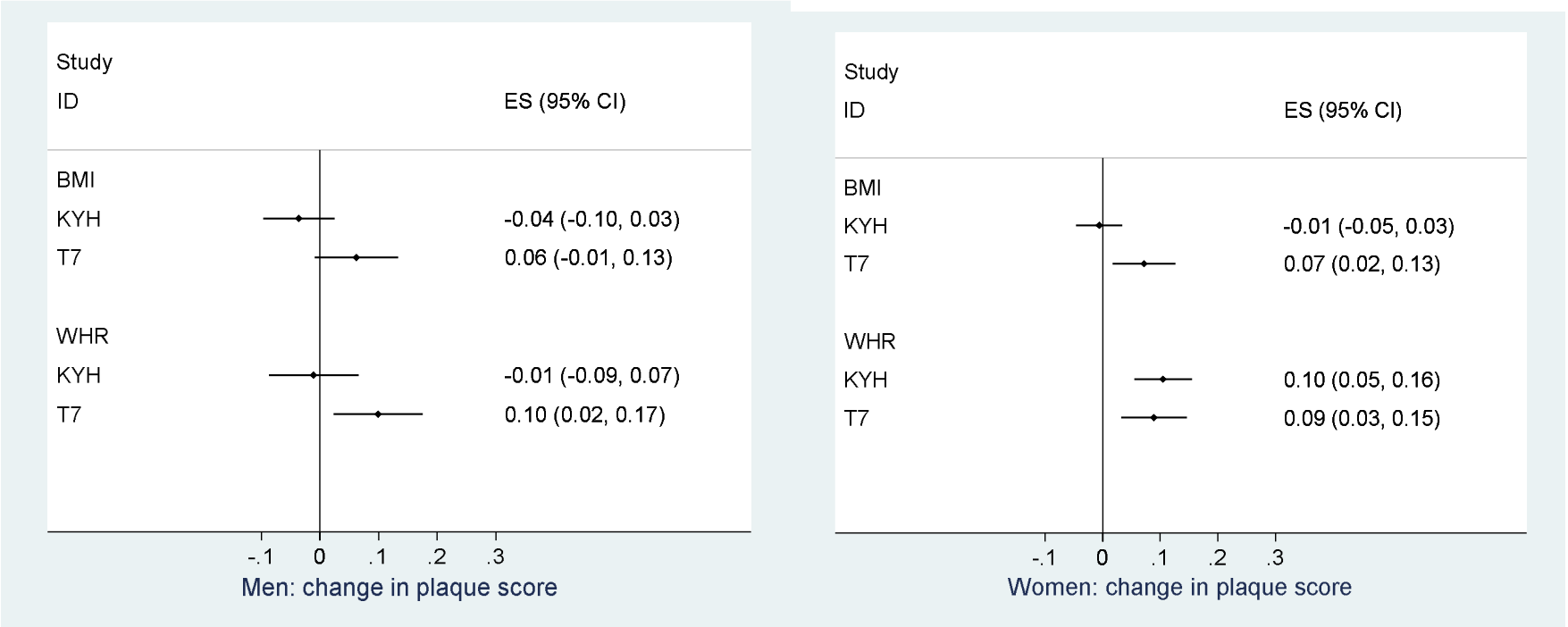
Supplementary figure 1A

Study-specific odds ratio for the prevalence of plaques for a 1 SD increase in each adiposity measure after the adjustment for age, smoking, and education (Model 2) (left: men, right: woman)



Supplementary figure 1B

Study-specific change in plaque score per 1 SD increase in each adiposity measure after the adjustment for age, smoking, and education (Model 2) (left: men, right: women)



STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5-6*
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	5
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	5
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	NA

Outcome data	15*	Report numbers of outcome events or summary measures	7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	16-17
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Supplementary
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.